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PATENT
674509-2020

FROMMER LAWRENCE & HAUG LLP
745 FIFTH AVENUE
NEW YORK, NEW YORK 10151
TEL. (212) 588-0800

Date: November 5, 1999

Re: 674509-2020

ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application (35 U.S.C. 111)
Washington, D.C. 20231

Sir:

With reference to the filing in the United States Patent and Trademark Office of a Continuation application of a PCT application, pursuant to 35 U.S.C. 111(a) in the name of:

Aksel BUCHTER-LARSEN and Ian MARCUSSEN

entitled: **A PROCESS FOR PREPARING AN ANTI-OXIDANT**

_ This is an application of a small entity under 37 CFR 1.9(f) and as only an unsigned Small Entity Verified Statement is enclosed, large entity fees have been paid, and a refund is respectfully requested upon the filing of the signed version of the Small Entity Verified Statement.

_ Small Entity Verified Statement is enclosed.

The following are enclosed:

X Specification (48 pages) and One Page of Abstract
X 0 Sheet(s) of Drawings
X 25 Claim(s) (including 8 independent claim(s))
_ This application contains a multiple dependent claim
X Oath or Declaration and Power of Attorney _ signed X unsigned
X Preliminary Amendment
X Our check for $1240.00, calculated as follows:

| | |
|---|---|
| Basic Fee, $760.00 ($380.00) | $ 760.00 |
| Number of Claims in excess of 20 at $18.00 ($9.00) each: | 90.00 |
| Number of Independent Claims in excess of 3 at $78.00 ($39.00) each: | 390.00 |
| Multiple Dependent Claim Fee at $260.00 ($130.00) | -0- |
| Total Filing Fee | $1240.00 |
| Assignment Recording Fee $40.00 | -0- |

X Pursuant to MPEP § 1895, enclosed is a copy of the PCT Request which is submitted as evidence that the instant continuation is copending with the PCT application and has at least one inventor in common therewith.
_ Certified copy of each of the following application(s) to substantiate the claim(s) for priority made in the Declaration:

| Application No. | Filed | In |
|---|---|---|
| PCT/IB98/00708 | 5/6/98 | PCT |
| 9709161.5 | 5/6/97 | Great Britain |

Jmm0583

09/423126
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Please charge any additional fees required for the filing of this application or credit any overpayment to Deposit Account No. 50-0320.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP
Attorneys for Applicant

By: Thomas J. Kowalski
Thomas J. Kowalski
Registration No. 32,147

09423126.110599

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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| Applicant(s) | : | Buchter-Larsen et al. |
| Filed | : | Herewith |
| Corresponding Int'l or Priority Applications | : | PCT/IB98/00708<br>British Appln. No. 9709161.5 |
| Priority Date | : | May 6, 1997 |
| For | : | A PROCESS FOR PREPARING AN ANTI-OXIDANT |

745 Fifth Avenue
New York, NY 10151

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Date of Deposit. November 5, 1999

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington, DC 20231

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## PRELIMINARY AMENDMENT

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of this U.S. application, please enter the following:

**IN THE CLAIMS:**

Please amend the claims as follows:

Claim 4, line 1: change "any of claims 1 to 3" to --claim 1--.

Claim 8, line 1: change "any of claims 4 to 7" to --claim 4--.

Claim 10, line 1: change "any of the preceding claims" to --claim 1--.

Claim 11, line 1: change "any of the preceding claims" to --claim 1--.

Claim 13, line 1: change "any of the preceding claims" to --claim 1--.

## REMARKS

This application includes multiple claim dependencies. The amendment removes or reduces the multiple claim dependencies, and the filing fee for this application was computed on the basis that no dependent claim depends from more than one preceding claim. No estoppel, surrender of subject matter, waiver, admission, or prejudice is intended by this amendment.

Entry of this amendment and an early examination on the merits are respectfully solicited.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: Thomas J. Kowalski

Thomas J. Kowalski
Reg. No. 32,147
(212) 588-0800

# A PROCESS OF PREPARING AN ANTI-OXIDANT

The present invention relates to a process of preparing an anti-oxidant.

An anti-oxidant prevents, inhibits or reduces the oxidation rate of an oxidisable medium. In particular, anti-oxidants are used for the preservation of food, especially when the food is or comprises a fat. Typical chemical anti-oxidants include aromatic amines, substituted phenols and sulphur compounds. Examples of anti-oxidants for food products are polyvinylpolypyrrolidone, dithiothreitol, sulphur dioxide, synthetic $\gamma$-tocopherol, $\delta$-tocopherol, L-ascorbic acid, sodium L-ascorbate, calcium L-ascorbate, ascorbyl palmitate, propyl gallate, octyl gallate, dodecyl gallate, lecithin, diphenylamine ethoxyquin and butylated hydroxytoluene. Two commonly used anti-oxidants are GRINDOX 142 (obtained from Danisco A/S) and GRINDOX 1029 (obtained from Danisco A/S).

Typically, anti-oxidants are added to foodstuffs, such as beverages.

For example, anti-oxidants are used in the preparation of alcoholic beverages such as beer, cider, ale etc.. In particular, there is a wide spread use of anti-oxidants in the preparation of wine. In this regard, Butzke and Bisson in Agro-Food-Industry Hi-Tech (July/August 1996 pages 26-30) present a review of wine manufacture.

According to Butzke and Bisson (*ibid*):

> "*Wine is the product of the natural fermentation of grape must or juice. In the case of red wine, the skins are present during the initial fermentation to allow extraction of pigment and important flavour and aroma constituents from the skin. The term "must" refers to the crushed whole grapes. In the case of white wine production, skins are removed prior to fermentation and only the juice is retained and processed. ....*

*Grapes are harvested and brought directly to the winery from the field. The grapes are then crushed at the winery and the must either transferred to a tank for fermentation (red wine) or pressed to separate juice from the skin and seeds (white wine). In this latter case, the juice is then transferred to a tank for fermentation. The tanks may either be inoculated with a commercial wine strain of Saccharomyces or allowed to undergo a natural or uninoculated fermentation. In a natural fermentation, Saccharomyces cells are greatly outnumbered by wild (non-Saccharomyces) yeast and bacteria at the beginning of fermentation. By the end of the fermentation Saccharomyces is the dominant and most often only organism isolateable. Inoculation with a commercial wine strain or with fermenting juice or must changes the initial ratio of the numbers of different microorganisms, allowing Saccharomyces to dominate the fermentation much earlier.*

*The metabolic activity of microorganisms in wine results in the production of aroma and flavour compounds some of which are highly objectionable to the consumer and all of which are distinct from the compounds responsible for the varietal character of the wine. .... Sulphur dioxide addition prevents chemical oxidation reactions and in this sense is an important stabilizer of the natural grape aroma and flavour. It may be added to the must or juice to preserve flavour, not necessarily as an antimicrobial agent. However, its antimicrobial activity must be considered when choosing a strain to be genetically modified for wine production."*

Hence, potentially harmful chemicals - such as sulphur dioxide - are used in wine manufacture.

The present invention seeks to overcome any problems associated with the prior art methods of preparing foodstuffs with antioxidants.

According to a first aspect of the present invention there is provided a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques.

According to a second aspect of the present invention there is provided a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared by use of a recombinant glucan lyase.

According to a third aspect of the present invention there is provided a medium prepared by the process according to the present invention.

Other aspects of the present invention include:

Use of anhydrofructose as an anti-oxidant for a medium comprising at least one other component, wherein the anhydrofructose is prepared *in situ* in the medium.

Use of anhydrofructose as a means for imparting or improving stress tolerance in a plant, wherein the anhydrofructose is prepared *in situ* in the plant.

Use of anhydrofructose as a means for imparting or improving the transformation of a grape, wherein the anhydrofructose is prepared *in situ* in the grape.

Use of anhydrofructose as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the anhydrofructose is prepared *in situ* in the foodstuff.

Use of anhydrofructose as a pharmaceutical in a foodstuff, wherein the anhydrofructose is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising anhydrofructose, wherein the anhydrofructose is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the anhydrofructose has been prepared *in situ* in the foodstuff.

Use of anhydrofructose as a nutraceutical in a foodstuff, wherein the anhydrofructose is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising anhydrofructose, wherein the anhydrofructose is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the anhydrofructose has been prepared *in situ* in the foodstuff.

Use of glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the glucan lyase is prepared *in situ* in the plant.

Use of glucan lyase as a means for imparting or improving the transformation of a grape, wherein the glucan lyase is prepared *in situ* in the grape.

Use of glucan lyase as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the glucan lyase is prepared *in situ* in the foodstuff.

Use of glucan lyase in the preparation of a pharmaceutical in a foodstuff, wherein the glucan lyase is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the antioxidant has been prepared *in situ* in the

foodstuff from a glucan lyase.

Use of glucan lyase in the preparation of a nutraceutical in a foodstuff, wherein the glucan lyase is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff from a glucan lyase.

Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the nucleotide sequence is expressed *in situ* in the plant.

Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving the transformation of a grape, wherein the nucleotide sequence is expressed *in situ* in the grape.

Use of a nucleotide sequence coding for a glucan lyase as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

Use of a nucleotide sequence coding for a glucan lyase as a means for creating a pharmaceutical in a foodstuff, wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff by means of a nucleotide sequence coding for a glucan lyase.

Use of a nucleotide sequence coding for a glucan lyase as a means for creating a nutraceutical in a foodstuff, wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff by means of a nucleotide sequence coding for a glucan lyase.

The term "nutraceutical" means a compound that is capable of acting as a nutrient (i.e. it is suitable for, for example, oral administration) as well as being capable of exhibiting a pharmaceutical effect and/or cosmetic effect.

In contrast to the usual practice of adding anti-oxidants media, such as foodstuffs, we have now found that particular anti-oxidants can be prepared *in situ* in the medium.

The *in situ* preparation of anti-oxidants is particularly advantageous in that less, or even no, additional anti-oxidants need be added to the medium, such as a food product.

The present invention is also believed to be advantageous as it provides a means of improving stress tolerance of plants.

The present invention is also advantageous as it provides a means for viably transforming grape.

The present invention is further advantageous in that it enables the levels of antioxidants in foodstuffs to be elevated. This may have beneficial health implications. In this regard, recent reports (e.g. Biotechnology Newswatch April 21 1997 "*Potent Antioxidants, as strong as those in fruit, found in coffee*" by Marjorie Shaffer) suggest that antioxidants have a pharmaceutical benefit, for example in preventing or suppressing cancer formation.

General *in situ* preparation of antioxidants in plants has been previously reviewed by Badiani *et al* in Agro-Food-Industry Hi-Tech (March/April 1996 pages 21-26). It is to be noted, however, that this review does not mention preparing *in situ* antioxidants from a glucan, let alone by use of a recombinant glucan lyase.

Preferably, the glucan comprises $\alpha$-1,4 links.

Preferably, the glucan is starch or a unit of starch.

Preferably, the glucan is a substrate for a recombinant enzyme such that contact of the glucan with the recombinant enzyme yields the anti-oxidant.

Preferably, the enzyme is a glucan lyase.

Preferably, the enzyme is an $\alpha$-1,4-glucan lyase.

Preferably, the enzyme comprises any one of the sequences shown as SEQ ID Nos 1-6, or a variant, homologue or fragment thereof.

Preferably, the enzyme is any one of the sequences shown as SEQ ID Nos 1-6.

Preferably, the enzyme is encoded by a nucleotide sequence comprising any one of the sequences shown as SEQ ID Nos 7-12, or a variant, homologue or fragment thereof.

Preferably, the enzyme is encoded by a nucleotide sequence having any one of the sequences shown as SEQ ID Nos 7-12.

Preferably, the anti-oxidant is anhydrofructose.

Preferably, the anti-oxidant is 1,5-D-anhydrofructose.

Preferably, the medium is, or is used in the preparation of, a foodstuff.

Preferably, the foodstuff is a beverage.

Preferably, the beverage is an alcoholic beverage.

Preferably, the beverage is a wine.

Preferably, the anti-oxidant is prepared *in situ* in the component and is then released into the medium.

Preferably, the component is a plant or a part thereof.

Preferably, the component is all or part of a cereal or a fruit.

Preferably, the component is all or part of a grape.

The medium may be used as or in the preparation of a foodstuff, which includes beverages. In the alternative, the medium may be for use in polymer chemistry. In this regard, the *in situ* generated anti-oxidants could therefore act as oxygen scavengers during, for example, the synthesis of polymers, such as the synthesis of bio-degradable plastic.

In accordance with the present invention, the anti-oxidant (preferably anhydrofructose) is prepared *in situ* in the medium. In other words, the antioxidant (preferably anhydrofructose) that is prepared *in situ* in the medium is used as an anti-oxidant in the medium. In one emdodiment, the antioxidant (preferably anhydrofructose) that is prepared *in situ* in the medium is used as the main anti-oxidant in the medium.

The term "*in situ* in the medium" as used herein includes the anti-oxidant being prepared by action of a recombinant enzyme expressed by the component on a glucan - which glucan is a substrate for the enzyme. The term also includes the anti-oxidant

being prepared by action of a recombinant enzyme expressed by the component on a glucan - which glucan is a substrate for the enzyme - within the component and the subsequent generation of the anti-oxidant. The term also includes the recombinant enzyme being expressed by the component and then being released into the medium, which enzyme acts on a glucan - which glucan is a substrate for the enzyme - present in the medium to form the anti-oxidant in the medium. The term also covers the presence or addition of another component to the medium, which component then expresses a recombinant nucleotide sequence which results in exposure of part or all of the medium to an anti-oxidant, which anti-oxidant may be a recombinant enzyme or a recombinant protein expressed and released by the other component, or it may be a product of a glucan - which glucan is a substrate for the enzyme - within the medium that has been exposed to the recombinant enzyme or the recombinant protein.

The term "by use of recombinant DNA techniques" as used herein includes the anti-oxidant being any obtained by use of a recombinant enzyme or a recombinant protein, which enzyme or protein acts on the glucan. The term also includes the anti-oxidant being any obtained by use of an enzyme or protein, which enzyme or protein acts on a recombinant glucan.

The term "starch" in relation to the present invention includes native starch, degraded starch, modified starch, including its components amylose and amylopectin, and the glucose units thereof.

The terms "variant", "homologue" or "fragment" in relation to the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has $\alpha$-glucan lyase activity, preferably having at least the same activity of any one of the enzymes shown as SEQ ID No. 1-6. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant enzyme has $\alpha$-glucan lyase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to any one of the sequences shown as SEQ ID No.s 1-6. More

preferably there is at least 95%, more preferably at least 98%, homology to any one of the sequences shown as SEQ ID No. 1-6.

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for an enzyme having $\alpha$-glucan lyase activity, preferably having at least the same activity of any one of the enzymes shown as SEQ ID No. 1-6. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for an enzyme having $\alpha$-glucan lyase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to any one of the sequences shown as SEQ ID No. 7-12. More preferably there is at least 95%, more preferably at least 98%, homology to any one of the sequences shown as SEQ ID No. 7-12.

The above terms are synonymous with allelic variations of the sequences.

The present invention also covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention.

The term "nucleotide" in relation to the present invention includes cDNA.

According to the present invention there is therefore provided a method of preparing *in situ* in an oxidisable medium an anti-oxidant. In a preferred embodiment, the anti-oxidant is anhydrofructose, more preferably 1,5-D-anhydrofructose. 1,5-D-anhydrofructose has been chemically synthesised (Lichtenthaler in Tetrahedron Letters Vol 21 pp 1429-1432). 1,5-D-anhydrofructose is further discussed in WO 95/10616, WO 95/10618 and GB-B-2294048.

The main advantages of using 1,5-D-anhydrofructose as an anti-oxidant are that it is a natural product, it is non-metabolisable, it is easy to manufacture, it is water-soluble, and it is generally non-toxic.

According to WO 95/10616, WO 95/10618 and GB-B-2294048, 1,5-D-anhydrofructose may be prepared by the enzymatic modification of substrates based on $\alpha$-1,4-glucan by use of the enzyme $\alpha$-1,4-glucan lyase. A typical $\alpha$-1,4-glucan based substrate is starch.

Today, starches have found wide uses in industry mainly because they are cheap raw materials. There are many references in the art to starch. For example, starch is discussed by Salisbury and Ross in Plant Physiology (Fourth Edition, 1991, Published by Wadsworth Publishing Company - especially section 11.7). In short, however, starch is one of the principal energy reserves of plants. It is often found in colourless plastids (amyloplasts), in storage tissue and in the stroma of chloroplasts in many plants. Starch is a polysaccharide carbohydrate. It comprises two main components: amylose and/or amylopectin. Both amylose and/or amylopectin consist of straight chains of $\alpha$(1,4)-linked glucose units (ie glycosyl residues) but in addition amylopectin includes $\alpha$(1,6) branched glucose units.

Some of the glucan lyases discussed in WO 95/10616 and WO 95/10618 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 1-4. Some of the glucan lyases discussed in GB-B-2294048 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 5-6.

Some of the nucleotide sequences coding for glucan lyases discussed in WO 95/10616 and WO 95/10618 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 7-10. Some of the nucleotide sequences coding for glucan lyases discussed in GB-B-2294048 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 11-12.

A further glucan lyase is discussed in WO 94/09122.

The recombinant nucleotide sequences coding for the enzyme may be cloned from sources such as a fungus, preferably *Morchella costata or Morchella vulgaris,* or from a fungally infected algae, preferably *Gracilariopsis lemaneiformis*, or from algae lone, preferably *Gracilariopsis lemaneiformis*.

In a preferred embodiment, the 1,5-D-anhydrofructose is prepared *in situ* by treating an $\alpha$-1,4-glucan with a recombinant $\alpha$-1,4-glucan lyase, such as any one of those presented as SEQ I.D. No.s 1-6.

Detailed commentary on how to prepare the enzymes shown as sequences SEQ I.D. No.s 1-6 may be found in the teachings of WO 95/10616, WO 95/10618 and GB-B-2294048. Likewise, detailed commentary on how to isolate and clone the nucleotide sequences SEQ I.D. No.s 7-12 may be found in the teachings of WO 95/10616, WO 95/10618 and GB-B-2294048.

If the glucan contains links other than and in addition to the $\alpha$-1,4- links the recombinant $\alpha$-1,4-glucan lyase can be used in conjunction with a suitable reagent that can break the other links - such as a recombinant hydrolase - preferably a recombinant glucanohydrolase.

General teachings of recombinant DNA techniques may be found in Sambrook,J., Fritsch, E.F., Maniatis T. (Editors) Molecular Cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory Press. New York 1989.

In order to express a nucleotide sequence, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press). If a prokaryotic host is used then the gene may need to be suitably modified before transformation - such as by removal of introns.

In one embodiment, the host organism can be of the genus *Aspergillus*, such as *Aspergillus niger*. A transgenic *Aspergillus* can be prepared by following the teachings of Rambosek, J. and Leach, J. 1987 (Recombinant DNA in filamentous fungi: Progress and Prospects. CRC Crit. Rev. Biotechnol. 6:357-393), Davis R.W. 1994 (Heterologous gene expression and protein secretion in *Aspergillus*. In: Martinelli S.D., Kinghorn J.R.( Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp 525-560), Ballance, D.J. 1991 (Transformation systems for Filamentous Fungi and an Overview of Fungal Gene structure. In: Leong, S.A., Berka R.M. (Editors) Molecular Industrial Mycology. Systems and Applications for Filamentous Fungi. Marcel Dekker Inc. New York 1991. pp 1-29) and Turner G. 1994 (Vectors for genetic manipulation. In: Martinelli S.D., Kinghorn J.R.( Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp. 641-666). However, the following commentary provides a summary of those teachings for producing transgenic *Aspergillus*.

For almost a century, filamentous fungi have been widely used in many types of industry for the production of organic compounds and enzymes. For example, traditional japanese koji and soy fermentations have used *Aspergillus sp*. Also, in this century *Aspergillus niger* has been used for production of organic acids particular citric acid and for production of various enzymes for use in industry.

There are two major reasons why filamentous fungi have been so widely used in industry. First filamentous fungi can produce high amounts of extracelluar products, for example enzymes and organic compounds such as antibiotics or organic acids. Second filamentous fungi can grow on low cost substrates such as grains, bran, beet pulp etc. The same reasons have made filamentous fungi attractive organisms as hosts for heterologous expression of recombinant enzymes according to the present invention.

In order to prepare the transgenic *Aspergillus*, expression constructs are prepared by inserting a requisite nucleotide sequence into a construct designed for expression in filamentous fungi.

Several types of constructs used for heterologous expression have been developed. These constructs can contain a promoter which is active in fungi. Examples of promoters include a fungal promoter for a highly expressed extracelluar enzyme, such as the glucoamylase promoter or the $\alpha$-amylase promoter. The nucleotide sequence can be fused to a signal sequence which directs the protein encoded by the nucleotide sequence to be secreted. Usually a signal sequence of fungal origin is used. A terminator active in fungi ends the expression system.

Another type of expression system has been developed in fungi where the nucleotide sequence can be fused to a smaller or a larger part of a fungal gene encoding a stable protein. This can stabilize the protein encoded by the nucleotide sequence. In such a system a cleavage site, recognized by a specific protease, can be introduced between the fungal protein and the protein encoded by the nucleotide sequence, so the produced fusion protein can be cleaved at this position by the specific protease thus liberating the protein encoded by the nucleotide sequence. By way of example, one can introduce a site which is recognized by a KEX-2 like peptidase found in at least some *Aspergilli*. Such a fusion leads to cleavage *in vivo* resulting in protection of the expressed product and not a larger fusion protein.

Heterologous expression in *Aspergillus* has been reported for several genes coding for bacterial, fungal, vertebrate and plant proteins. The proteins can be deposited intracellularly if the nucleotide sequence is not fused to a signal sequence. Such proteins will accumulate in the cytoplasm and will usually not be glycosylated which can be an advantage for some bacterial proteins. If the nucleotide sequence is equipped with a signal sequence the protein will accumulate extracelluarly.

With regard to product stability and host strain modifications, some heterologous proteins are not very stable when they are secreted into the culture fluid of fungi.

Most fungi produce several extracelluar proteases which degrade heterologous proteins. To avoid this problem special fungal strains with reduced protease production have been used as host for heterologous production.

For the transformation of filamentous fungi, several transformation protocols have been developed for many filamentous fungi (Ballance 1991, *ibid*). Many of them are based on preparation of protoplasts and introduction of DNA into the protoplasts using PEG and $Ca^{2+}$ ions. The transformed protoplasts then regenerate and the transformed fungi are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as *argB*, *trpC*, *niaD* and *pyrG*, antibiotic resistance markers such as benomyl resistance, hygromycin resistance and phleomycin resistance. A commonly used transformation marker is the *amdS* gene of *A. nidulans* which in high copy number allows the fungus to grow with acrylamide as the sole nitrogen source.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The species *Saccharomyces cerevisiae* has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey *et al* (1987, Yeast Biotechnology, D R Berry *et al*, eds, pp 401-429, Allen and Unwin, London) and by King *et al* (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic *Saccharomyces*, expression constructs are prepared by inserting the nucleotide sequence into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic *Saccharomyces* can be prepared by following the teachings of Hinnen *et al* (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H *et al* (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, eg G418.

Another host organism is a plant. In this regard, the art is replete with references for preparing transgenic plants. Two documents that provide some background commentary on the types of techniques that may be employed to prepare transgenic plants are EP-B-0470145 and CA-A-2006454 - some of which commentary is

presented below.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a recombinant nucleotide sequence and which is capable of introducing the nucleotide sequence into the genome of an organism, such as a plant, and wherein the nucleotide sequence is capable of preparing *in situ* an anti-oxidant.

The vector system may comprise one vector, but it can comprise at least two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An et al. (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* (An et al. (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208).

Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above.

The nucleotide sequence of the present invention should preferably be inserted into

the Ti-plasmid between the border sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appear to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant, then the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct. Preferably, the vector system is an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof, as these plasmids are well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct or vector of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli.*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli.* it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the first nucleotide sequence or construct of the invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the invention, which DNA is subsequently transferred into the plant cell to be modified.

As reported in CA-A-2006454, a large number of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR322, the pUC series, the M13 mp series, pACYC 184 etc. In this way, the promoter or nucleotide or construct of the present invention can be introduced into a suitable restriction position

in the vector. -The contained plasmid is used for the transformation in *E.coli*. The *E.coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered and then analysed - such as by any one or more of the following techniques: sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted or selectively amplified by PCR techniques and connected with the next DNA sequence. Each sequence can be cloned in the same or different plasmid.

After each introduction method of the nucleotide sequence or construct or vector according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An et al., EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. et al. (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be done on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the first nucleotide sequence or the construct, a plant to be infected is wounded, e.g. by cutting the plant with a razor or puncturing the plant with a needle or rubbing the

plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium.

When plant cells are constructed, these cells are grown and, optionally, maintained in a medium according to the present invention following well-known tissue culturing methods - such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc, but wherein the culture medium comprises a component according to the present invention. Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting the transformed shoots and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

Reference may even be made to Spngstad *et al* (1995 Plant Cell Tissue Organ Culture **40** pp 1-15) as these authors present a general overview on transgenic plant construction.

In one embodiment, the plant is a grapevine. There are a number of teachings in the art on how to prepare transformed grapevines. For example, reference may be made to Baribault *et al* (J Exp Bot 41 (229) 1990 1045-1050), Baribault *et al* (Plant Cell Rep 8 (3) 1989 137-140), Scorza *et al* (J Am Soc Horticultural Science 121 (4) 1996 616-619), Kikkert *et al* (Plant Cell Reports 15 (5) 1996 311-316), Golles *et al* (Acta Hortic 1997 vol 447 Number: Horticultural Biotechnology in Vitro Culture and Breeding Pages 265-275), Gray and Scorza (WO-A-97/49277) and Simon Robinson *et al* (Conference abstracts and paper presented in Biotechnology - Food and Health for the 21st Century, Adelaide, Australia, 1998). By way of example Robinson *et al* (*ibid*) disclose a method for transforming grapevine wherein somatic embryos are induced on callus formed from another tissue and *Agrobacterium* infection is used to transfer target genes into the embryo tissue.

Further reference may be made to the teachings of Andrew Walker in Nature Biotechnology (Vol 14, May 1996, page 582) who states that:

> *"The grape, one of the most important fruit plants in the world, has been difficult to engineer because of its high levels of tannins and phenols, which interfere with cell culture and transformation; the compounds oxidize quickly and promote the decay of grape cells."*

In that same edition of Nature Biotechnology, Perl *et al* (pages 624-628) report on the use of the combination of polyvinylpolypyrrolidone and dithiothreitol to improve the viability of grape transformation during *Agrobacterium* infection.

Hence, the present invention provides an alternative means for transforming grape. In this regard, the antioxidant that is prepared *in situ* by a grape transformed in accordance with the present invention improves the viability of grape transformation during *Agrobacterium* infection.

Thus, according to one aspect of the present invention, there is provided the use of an antioxidant prepared *in situ* in order to effectively transform a grape.

In some instances, it is desirable for the recombinant enzyme or protein to be easily secreted into the medium to act as or to generate an anti-oxidant therein. In such cases, the DNA encoding the recombinant enzyme is fused to *inter alia* an appropriate signal sequence, an appropriate promoter and an appropriate terminator from the chosen host.

For example, for expression in *Aspergillus niger* the gpdA (from the Glyceraldehyde-3-phosphate dehydrogenase gene of *Aspergillus nidulans*) promoter and signal sequence is fused to the 5' end of the DNA encoding the mature lyase. The terminator sequence from the *A. niger* trpC gene is placed 3' to the gene (Punt, P.J. et al 1991 - (1991): J. Biotech. 17, 19-34). This construction is inserted into a vector containing a replication origin and selection origin for *E. coli* and a selection marker

for *A. niger*. -Examples of selection markers for *A. niger* are the amdS gene, the argB gene, the pyrG gene, the hygB gene, the BmlR gene which all have been used for selection of transformants. This plasmid can be transformed into *A. niger* and the mature lyase can be recovered from the culture medium of the transformants. Eventually the construction could be transformed into a protease deficient strain to reduce the proteolytic degradation of the lyase in the medium (Archer D.B. et al 1992 -Biotechnol. Lett. 14, 357-362).

In addition, and as indicated above, aside from using *Aspergillus niger* as the host, there are other industrial important microorganisms which could be used as expression systems. Examples of these other hosts include: *Aspergillus oryzae, Aspergillus sp., Trichoderma sp., Saccharomyces cerevisiae, Kluyveromyces sp., Hansenula sp., Pichia sp., Bacillus subtilis, B. amyloliquefaciens, Bacillus sp., Streptomyces sp.* or *E. coli*.

In accordance with the present invention, a suitable marker or selection means may be introduced into the host that is to be transformed with the nucleotide sequence. Examples of suitable markers or selection means are described in any one of WO-A-93/05163, WO-A-94/20627, GB patent application No. 9702591.0 (filed 7 February 1997), GB patent application No. 9702576.1 (filed 7 February 1997), GB patent application No. 9702539.9 (filed 7 February 1997), GB patent application No. 9702510.0 (filed 7 February 1997) and GB patent application No. 9702592.8 (filed 7 February 1997).

In summation, the present invention relates to a process comprising preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques and/or the anti-oxidant is prepared by use of a recombinant glucan lyase.

In a preferred embodiment, the present invention relates to a process a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of a recombinant glucan lyase.

In a more preferred embodiment, the present invention relates to a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; wherein the anti-oxidant is prepared from a glucan by use of a recombinant glucan lyase; and wherein the anti-oxidant is anhydro-fructose.

The present invention will now be described only by way of example.

## TRANSGENIC GRAPE

Transformed grapes are prepared following the teachings of Perl *et al* (*ibid*) but wherein the use of the combination of polyvinylpolypyrrolidone and dithiothreitol is optional. In these studies, the grapes are transformed with any one of the nucleotide sequences presented as SEQ ID No. 7-12. The transformation leads to *in situ* preparation of 1,5-D-anhydrofructose. The transformed grapes are beneficial for one or more of the reasons mentioned earlier.

Details on these studies are as follows.

### Tissue-culture systems for transformation studies

The long term somatic embryogenic callus culture is developed from the vegetative tissues of anthers of *Vitis vinifera* CV Superior Seedless. Methods for another culture, induction of somatic embryogenesis and maintenance of embryogenic cultures, are previously described (Perl *et al*, 1995, Plant Sci **104**: 193-200). Briefly, embryogenic calli are maintained on solidified (0.25% gelrite) MS medium (Murashige and Skoog, 1962, Physiol Plant **15**: 473-497) supplemented with 6%

sucrose, 2 mg/L 2,4-diclorophenoxyacetic acid (2,4-D), 5 mg/L Indole-3-aspartic acid (IASP), 0.2 mg/L 6-benzyladenine (BAP) and 1 mg/L abscisic acid (ABA). Proembryogenic calli are induced by transferring the calli to MS medium supplemented with the same phytohormones, but 2,4-D is substituted with 2 mg/L 2-naphthoxyacetic acid (NOA). This stage is used for transformation experiments.

### *Agrobacterium* strains

For studying the sensitivity of grape embryogenic calli to the presence of different *Agrobacterium* strains, or for stable transformation experiments, cocultivation is attempted using the following *A tumefaciens* strains: EHA 101-p492 (Perl *et al*. 1993, Bio/Technology **11**:715-718); LBA 4404-pGPTV (Becker *et al*, 1992, Plant Mol Biol **20**: 1195-1197); and GVE 3101-pPCV91 (Vancanneyt *et al*, 1990, Mol Gen Genet **220**: 245-250). These strains contain the binary vectors conferring resistance to kanamycin (*nptII*), basta (*bar*) and hygromycin (*hpt*), respectively, all under the control of the nopalin-synthase (NOS) promoter and terminator. Bacteria are cultured with the proper antibiotics in liquid LB medium for 24 hours at 28°C at 200 rpm.

### Cocultivation

For studying the sensitivity of grape embryogenic calli to different *Agrobacterium* strains, bacterial cultures with different optical densities (0.1-0.7 at 630 nm) are prepared from an overnight culture of *Agrobacterium* strains. Bacteria are centrifuged 5 minutes, 5000 rpm and resuspended in antibiotic free McCown's Woody Plant Medium (WPM) (Lloyd and McCown, 1981, Int Plant Prop Soc Proc **30**: 421-427). Three grams fresh weight of embryogenic calli (7 days after transfer to NOA containing medium) are resuspended in 10 ml of overnight cultured bacterial suspensions for 5 minutes, dry blotted and transferred to Petri dishes containing regeneration medium [basal WPM medium supplemented with thidiazuron (TDZ) (0.5 mg/L), Zeatin riboside (ZR) (0.5 mg/L), and sucrose (3%)]. The regeneration medium is solidified with gelrite (0.25% w/v) and the calli, after initial drainage of excess bacteria, are cocultivated in the dark at 25°C for different times (5 minutes

up to 7 days). For stable transformation experiments, inoculum (OD 0.6 at 630 nm) is prepared from an overnight culture of LBA 4404 or GVE 3101. Bacteria are centrifuged 5 minutes, 5000 rpm and resuspended in antibiotic-free WPM medium. Embryogenic calli (3g fresh weight) are resuspended in 10 ml of bacteria for 5 minutes, dry blotted and transferred to Petri dishes containing solidified (0.25% w/v) gelrite regeneration medium supplemented with different antioxidants. The calli are cocultivated for 48 hours in the dark at 25°C.

**Selective culture**

Following 48 hours of cocultivation, the embryogenic callus is maintained in the dark for 7 days on antioxidant containing regeneration medium. Subsequently, the calli are collected on a sterile metal screen and transferred to fresh WPM regeneration medium at 25°C under 40 $\mu E/m^2/s$ (white fluorescent tubes). All regeneration media are supplemented with 400 mg/L claforan, 1.5 g/L malt extract and different selectable markers: kanamycin (50-500 mg/L), hygromycin (15 mg/L) and Basta (1-10 mg/L). Periodic increases in hygromycin concentration are used. The putative transformed calli are cultured on regeneration medium supplemented with 15 mg/L hygromycin. Every two weeks the regenerating calli are transferred to fresh medium supplemented with 20 and 25 mg/L hygromycin respectively. Control, untransformed grape calli are also cultured on selective media and are periodically exposed to increasing hygromycin concentrations. Green adventitious embryos, which developed on calli cultured for 8-10 weeks on selective regeneration medium, are transferred to germination medium. Embryo germination, rooting and subsequent plantlet development are induced on WPM as described (Perl *et al*, 1995, Plant Sci **104**: 193-200), supplemented with 25 mg/L hygromycin or 10 mg/L basta. Conversion of vitrified abnormal plantlets into normal-looking grape plantlets are obtained using solidified WPM medium supplemented with 0.1 mg/L NAA as described (Perl *et al*, 1995, Plant Sci **104**: 193-200).

## TRANSGENIC POTATOES

General teachings on potato transformation may be found in our copending patent applications PCT/EP96/03053, PCT/EP96/03052 and PCT/EP94/01082 (the contents of each of which are incorporated herein by reference).

For the present studies, the following protocol is adopted.

### Plasmid construction

The disarmed *Agrobacterium tumefaciens* strain LBA 4404, containing the helper *vir* plasmid pRAL4404 (Hoekema *et al*, 1983 Nature **303** pp 179-180), is cultured on YMB agar ($K_2HPO_4.3H_2O$ 660 mg $l^{-1}$, $MgSO_4$ 200 mg $l^{-1}$, NaCl 100 mg $l^{-1}$, mannitol 10 g $l^{-1}$, yeast extract 400 mg $l^{-1}$, 0.8% w/v agar, pH 7.0) containing 100 mg $l^{-1}$ rifampicin and 500 mg $l^{-1}$ streptomycin sulphate. Transformation with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB*' (which correspond to each of pVICTOR IV GNG E35S *nagB* IV2 or pVICTOR IV GNG rbc *nagB* IV2 or pVICTOR IV GNG E35S *nagB* but wherein each of those plasmids also contains any one of the nucleotide sequences shown as SEQ ID No.s. 7-12 operatively linked to a functional promoter) is accomplished using the freeze-thaw method of Holters *et al* (1978 Mol Gen Genet **163** 181-187) and transformants are selected on YMB agar containing 100 mg $l^{-1}$ rifampicin and 500 mg $l^{-1}$ streptomycin, and 50 mg $l^{-1}$ gentamycin sulphate.

### Transformation of plants

Shoot cultures of *Solanum tuberosum* cv Saturna are maintained on LS agar containing Murashige Skoog basal salts (Sigma M6899) (Murashige and Skoog, 1965, Physiol Plant **15** 473-497) with 2 $\mu$M silver thiosulphate, and nutrients and vitamins as described by Linsmaier and Skoog (1965 Physiol Plant **18** 100-127). Cultures are maintained at 25°C with a 16h daily photoperiod. After approximately 40 days, subculturing is performed during which leaves are removed, and the shoots cut into

mononodal segments of approximately 8 mm length.

Shoot cultures of approximately 40 days maturity (5-6 cm height) are cut into 8 mm internodal segments which are placed into liquid LS-medium containing *Agrobacterium tumefaciens* transformed with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB'* ($A_{660}$ = 0.5, pathlength 1 cm). Following incubation at room temperature for 30 minutes, the segments are dried by blotting on to sterile filter paper and transferred to LS agar (0.8% w/v containing 2 mg $l^{-1}$ 2,4-D and 500 $\mu$g $l^{-1}$ trans-zeatin. The explants are covered with filter paper, moistened with LS medium, and covered with a cloth for three days at 25°C. Following this treatment, the segments are washed with liquid LS medium containing 800 mg $l^{-1}$ carbenicillin, and transferred on to LS agar (0.8% w/v) containing 1 mg $l^{-1}$ trans-zeatin, 100 $\mu$g $l^{-1}$ gibberellic acid (GA3), with sucrose (eg 7.5 g $l^{-1}$) and glucosamine (eg 2.5 g $l^{-1}$) as the selection agent.

The segments are sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continues for 3-4 months.

Rooting of regenerated shoots

The regenerated shoots are transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

The transgenic plants may be verified by performing a GUS assay on the co-introduced $\beta$-glucuronidase gene according to Hodal, L. *et al*. (Pl. Sci. (1992), 87: 115-122).

Alternatively, the transgenic genotype of the regenerated shoot may be verified by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154).

Transfer to soil

The newly rooted plants (height approx. 2-3 cms) are transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/$m^2$/sec). When the plants are well established they are transferred to the greenhouse, where they are grown until tubers had developed and the upper part of the plants are senescing.

Harvesting

The potatoes are harvested after about 3 months.

## TRANSGENIC MAIZE PLANTS

### Introduction

Since the first publication of production of transgenic plants in 1983 (Leemans, 1993 Biotechnology **11** s22), there have been numerous publications of production of transgenic plants including especially dicotyledon crop plants.

Until very recently there are very few reports on successful production of transgenic monocotyledononary crop plants. This relatively slow development within monocots are due to two causes. Firstly, until the early 1980s, efficient regeneration of plants from cultured cells and tissues of monocots had proven very difficult. This problem is ultimately solved by the culture of explants from immature and embryogenic tissue, which retain their morphogenic potential on nutrient media containing plant growth regulators. Secondly, the monocots are not a natural host for *Agrobacterium tumefaciens*, meaning that the successful developed techniques within the dicots using their natural vector *Agrobacterium tumefaciens* is unsuccessful for many years in the monocots.

Nevertheless, it is now possible to successfully transformation and produce fertile

transgenic plants of maize using methods such as: (1) Silicon Carbide Whiskers; (2) Particle Bombardment; (3) DNA Uptake by PEG treated protoplast; or (4) DNA Uptake in Electroporation of Tissue. Each of these methods - which are reviewed by Thompson (1995 Euphtytica **85** pp 75-80) - may be used to prepare *inter alia* transgenic maize according to the present invention.

In particular, the particle Gun method has been successfully used for the transformation of monocots. However, EP-A-0604662 reports on a different method of transforming monocotyledons. The method comprises transforming cultured tissues of a monocotyledon under or after dedifferentiation with *Agrobacterium* containing a super binary vector as a selectable marker a hygromycin-resistant gene is used. Production of transgenic calli and plant is demonstrated using the hygromycin selection. This method may be used to prepare *inter alia* transgenic maize according to the present invention.

Subsequent to the method of EP-A-0604662, EP-A-0672752 reports on non-dedifferentiated immature embryos. In this regard, both hygromycin-resistance and PPT-resistance genes are used as the selectable marker, with PPT giving rise to 10% or more independent transformed plants. This method may be used to prepare *inter alia* transgenic maize according to the present invention.

To date, it would appear that transgenic maize plants can be successfully produced from easily-culturable varieties - such as the inbred line A188. In this regard, see the teachings of Ishida *et al* (1996 Nature Biotechnology **14** pp 745-750). The method disclosed by these workers may be used to prepare *inter alia* transgenic maize according to the present invention.

Vasil (1996 Nature Biotechnology **14** pp 702-703) presents a further review article on transformation of maize. Even though it is possible to prepare transformed maize by use of, for example, particle Gun mediated transformation, for the present studies the following protocol is adopted.

**Plasmid construction**

The disarmed *Agrobacterium tumefaciens* strain LBA 4404, containing the helper *vir* plasmid pRAL4404 (Hoekema *et al*, 1983 Nature **303** pp 179-180), is cultured on YMB agar ($K_2HPO_4.3H_2O$ 660 mg $l^{-1}$, $MgSO_4$ 200 mg $l^{-1}$, NaCl 100 mg $l^{-1}$, mannitol 10 g $l^{-1}$, yeast extract 400 mg $l^{-1}$, 0.8% w/v agar, pH 7.0) containing 100 mg $l^{-1}$ rifampicin and 500 mg $l^{-1}$ streptomycin sulphate. Transformation with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB'* is accomplished using the freeze-thaw method of Holters *et al* (1978 Mol Gen Genet **163** 181-187) and transformants are selected on YMB agar containing 100 mg $l^{-1}$ rifampicin and 500 mg $l^{-1}$ streptomycin, and 50 mg $l^{-1}$ gentamycin sulphate.

**Isolation and cocultivation of explants**

Immature embryos of, for example, maize line A188 of the size between 1.5 to 2.5 mm are isolated and cocultivated with *Agrobacterium tumefaciens* strain LBA 4404 in N6-AS for 2-3 days at 25°C under illumination. Thereafter, the embryos are washed with sterilized water containing 250 mg/l of cefotaxime and transferred to an LS medium and 250 mg/l cefotaxime and glucosamine in concentrations of up to 100 mg/l (the medium is hereafter called LSS1).

**Conditions for the selection of transgenic plants**

The explants are cultured for three weeks on LSS1 medium and then transferred to an LS medium containing glucosamine and cefotaxime. After three weeks on this medium, green shoots are isolated.

**Rooting of transformed shoots**

Transformed shoots are transferred to an MS medium containing 2 mg/l for rooting. After four weeks on this medium, plantlets are transferred to pots with sterile soil for acclimatisation.

## TRANSGENIC GUAR PLANTS

Transformation of guar cotyledonary explants is performed according to Joersbo and Okkels (PCT/DK95/00221) using *Agrobacterium tumefaciens* LBA4404 harbouring a suitable plasmid.

Other plants may be transformed in accordance with the present invention, such as other fruits, other vegetables, and other plants such as coffee plants, tea plants etc.

Other modifications of the present invention will be apparent to those skilled in the art.

SEQUENCES

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME DANISCO A/S
(B) STREET: LANGEBROGADE 1
(C) CITY. COPENHAGEN
(D) STATE: COPENHAGEN K
(E) COUNTRY: DENMARK
(F) POSTAL CODE (ZIP): DK-1001

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1088 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION. SEQ ID NO: 1:

```
Met Phe Ser Thr Leu Ala Phe Val Ala Pro Ser Ala Leu Gly Ala Ser
1               5                   10                  15
Thr Phe Val Gly Ala Glu Val Arg Ser Asn Val Arg Ile His Ser Ala
            20                  25                  30
Phe Pro Ala Val His Thr Ala Thr Arg Lys Thr Asn Arg Leu Asn Val
        35                  40                  45
Ser Met Thr Ala Leu Ser Asp Lys Gln Thr Ala Thr Ala Gly Ser Thr
    50                  55                  60
Asp Asn Pro Asp Gly Ile Asp Tyr Lys Thr Tyr Asp Tyr Val Gly Val
65                  70                  75                  80
Trp Gly Phe Ser Pro Leu Ser Asn Thr Asn Trp Phe Ala Ala Gly Ser
                85                  90                  95
Ser Thr Pro Gly Gly Ile Thr Asp Trp Thr Ala Thr Met Asn Val Asn
            100                 105                 110
Phe Asp Arg Ile Asp Asn Pro Ser Ile Thr Val Gln His Pro Val Gln
        115                 120                 125
Val Gln Val Thr Ser Tyr Asn Asn Asn Ser Tyr Arg Val Arg Phe Asn
    130                 135                 140
Pro Asp Gly Pro Ile Arg Asp Val Thr Arg Gly Pro Ile Leu Lys Gln
145                 150                 155                 160
Gln Leu Asp Trp Ile Arg Thr Gln Glu Leu Ser Glu Gly Cys Asp Pro
                165                 170                 175
Gly Met Thr Phe Thr Ser Glu Gly Phe Leu Thr Phe Glu Thr Lys Asp
            180                 185                 190
Leu Ser Val Ile Ile Tyr Gly Asn Phe Lys Thr Arg Val Thr Arg Lys
        195                 200                 205
Ser Asp Gly Lys Val Ile Met Glu Asn Asp Glu Val Gly Thr Ala Ser
    210                 215                 220
Ser Gly Asn Lys Cys Arg Gly Leu Met Phe Val Asp Arg Leu Tyr Gly
225                 230                 235                 240
Asn Ala Ile Ala Ser Val Asn Lys Asn Phe Arg Asn Asp Ala Val Lys
                245                 250                 255
Gln Glu Gly Phe Tyr Gly Ala Gly Glu Val Asn Cys Lys Tyr Gln Asp
            260                 265                 270
Thr Tyr Ile Leu Glu Arg Thr Gly Ile Ala Met Thr Asn Tyr Asn Tyr
        275                 280                 285
Asp Asn Leu Asn Tyr Asn Gln Trp Asp Leu Arg Pro Pro His His Asp
    290                 295                 300
Gly Ala Leu Asn Pro Asp Tyr Tyr Ile Pro Met Tyr Tyr Ala Ala Pro
305                 310                 315                 320
Trp Leu Ile Val Asn Gly Cys Ala Gly Thr Ser Glu Gln Tyr Ser Tyr
                325                 330                 335
Gly Trp Phe Met Asp Asn Val Ser Gln Ser Tyr Met Asn Thr Gly Asp
            340                 345                 350
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Thr Thr Trp Asn Ser Gly Gln Glu Asp Leu Ala Tyr Met Gly Ala Gln
355 360 365
Tyr Gly Pro Phe Asp Gln His Phe Val Tyr Gly Ala Gly Gly Gly Met
370 375 380
Glu Cys Val Val Thr Ala Phe Ser Leu Leu Gln Gly Lys Glu Phe Glu
385 390 395 400
Asn Gln Val Leu Asn Lys Arg Ser Val Met Pro Pro Lys Tyr Val Phe
405 410 415
Gly Phe Phe Gln Gly Val Phe Gly Thr Ser Ser Leu Leu Arg Ala His
420 425 430
Met Pro Ala Gly Glu Asn Asn Ile Ser Val Glu Glu Ile Val Glu Gly
435 440 445
Tyr Gln Asn Asn Asn Phe Pro Phe Glu Gly Leu Ala Val Asp Val Asp
450 455 460
Met Gln Asp Asn Leu Arg Val Phe Thr Thr Lys Gly Glu Phe Trp Thr
465 470 475 480
Ala Asn Arg Val Gly Thr Gly Gly Asp Pro Asn Asn Arg Ser Val Phe
485 490 495
Glu Trp Ala His Asp Lys Gly Leu Val Cys Gln Thr Asn Ile Thr Cys
500 505 510
Phe Leu Arg Asn Asp Asn Glu Gly Gln Asp Tyr Glu Val Asn Gln Thr
515 520 525
Leu Arg Glu Arg Gln Leu Tyr Thr Lys Asn Asp Ser Leu Thr Gly Thr
530 535 540
Asp Phe Gly Met Thr Asp Asp Gly Pro Ser Asp Ala Tyr Ile Gly His
545 550 555 560
Leu Asp Tyr Gly Gly Gly Val Glu Cys Asp Ala Leu Phe Pro Asp Trp
565 570 575
Gly Arg Pro Asp Val Ala Glu Trp Trp Gly Asn Asn Tyr Lys Lys Leu
580 585 590
Phe Ser Ile Gly Leu Asp Phe Val Trp Gln Asp Met Thr Val Pro Ala
595 600 605
Met Met Pro His Lys Ile Gly Asp Asp Ile Asn Val Lys Pro Asp Gly
610 615 620
Asn Trp Pro Asn Ala Asp Asp Pro Ser Asn Gly Gln Tyr Asn Trp Lys
625 630 635 640
Thr Tyr His Pro Gln Val Leu Val Thr Asp Met Arg Tyr Glu Asn His
645 650 655
Gly Arg Glu Pro Met Val Thr Gln Arg Asn Ile His Ala Tyr Thr Leu
660 665 670
Cys Glu Ser Thr Arg Lys Glu Gly Ile Val Glu Asn Ala Asp Thr Leu
675 680 685
Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg Gly Gly Tyr Ile Gly
690 695 700
Asn Gln His Phe Gly Gly Met Trp Val Gly Asp Asn Ser Thr Thr Ser
705 710 715 720
Asn Tyr Ile Gln Met Met Ile Ala Asn Asn Ile Asn Met Asn Met Ser
725 730 735
Cys Leu Pro Leu Val Gly Ser Asp Ile Gly Gly Phe Thr Ser Tyr Asp
740 745 750
Asn Glu Asn Gln Arg Thr Pro Cys Thr Gly Asp Leu Met Val Arg Tyr
755 760 765
Val Gln Ala Gly Cys Leu Leu Pro Trp Phe Arg Asn His Tyr Asp Arg
770 775 780
Trp Ile Glu Ser Lys Asp His Gly Lys Asp Tyr Gln Glu Leu Tyr Met
785 790 795 800
Tyr Pro Asn Glu Met Asp Thr Leu Arg Lys Phe Val Glu Phe Arg Tyr
805 810 815
Arg Trp Gln Glu Val Leu Tyr Thr Ala Met Tyr Gln Asn Ala Ala Phe
820 825 830
Gly Lys Pro Ile Ile Lys Ala Ala Ser Met Tyr Asn Asn Asp Ser Asn
835 840 845
Val Arg Arg Ala Gln Asn Asp His Phe Leu Leu Gly Gly His Asp Gly
850 855 860
Tyr Arg Ile Leu Cys Ala Pro Val Val Trp Glu Asn Ser Thr Glu Arg
865 870 875 880

Glu Leu Tyr Leu Pro Val Leu Thr Gln Trp Tyr Lys Phe Gly Pro Asp
885 890 895

Phe Asp Thr Lys Pro Leu Glu Gly Ala Met Asn Gly Gly Asp Arg Ile
900 905 910

Tyr Asn Tyr Pro Val Pro Gln Ser Glu Ser Pro Ile Phe Val Arg Glu
915 920 925

Gly Ala Ile Leu Pro Thr Arg Tyr Thr Leu Asn Gly Glu Asn Lys Ser
930 935 940

Leu Asn Thr Tyr Thr Asp Glu Asp Pro Leu Val Phe Glu Val Phe Pro
945 950 955 960

Leu Gly Asn Asn Arg Ala Asp Gly Met Cys Tyr Leu Asp Asp Gly Gly
965 970 975

Val Thr Thr Asn Ala Glu Asp Asn Gly Lys Phe Ser Val Val Lys Val
980 985 990

Ala Ala Glu Gln Asp Gly Gly Thr Glu Thr Ile Thr Phe Thr Asn Asp
995 1000 1005

Cys Tyr Glu Tyr Val Phe Gly Gly Pro Phe Tyr Val Arg Val Arg Gly
1010 1015 1020

Ala Gln Ser Pro Ser Asn Ile His Val Ser Ser Gly Ala Gly Ser Gln
1025 1030 1035 1040

Asp Met Lys Val Ser Ser Ala Thr Ser Arg Ala Ala Leu Phe Asn Asp
1045 1050 1055

Gly Glu Asn Gly Asp Phe Trp Val Asp Gln Glu Thr Asp Ser Leu Trp
1060 1065 1070

Leu Lys Leu Pro Asn Val Val Leu Pro Asp Ala Val Ile Thr Ile Thr
1075 1080 1085

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1091 amino acids
(B) TYPE. amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Tyr Pro Thr Leu Thr Phe Val Ala Pro Ser Ala Leu Gly Ala Arg
1 5 10 15

Thr Phe Thr Cys Val Gly Ile Phe Arg Ser His Ile Leu Ile His Ser
20 25 30

Val Val Pro Ala Val Arg Leu Ala Val Arg Lys Ser Asn Arg Leu Asn
35 40 45

Val Ser Met Ser Ala Leu Phe Asp Lys Pro Thr Ala Val Thr Gly Gly
50 55 60

Lys Asp Asn Pro Asp Asn Ile Asn Tyr Thr Thr Tyr Asp Tyr Val Pro
65 70 75 80

Val Trp Arg Phe Asp Pro Leu Ser Asn Thr Asn Trp Phe Ala Ala Gly
85 90 95

Ser Ser Thr Pro Gly Asp Ile Asp Asp Trp Thr Ala Thr Met Asn Val
100 105 110

Asn Phe Asp Arg Ile Asp Asn Pro Ser Phe Thr Leu Glu Lys Pro Val
115 120 125

Gln Val Gln Val Thr Ser Tyr Lys Asn Asn Cys Phe Arg Val Arg Phe
130 135 140

Asn Pro Asp Gly Pro Ile Arg Asp Val Asp Arg Gly Pro Ile Leu Gln
145 150 155 160

Gln Gln Leu Asn Trp Ile Arg Lys Gln Glu Gln Ser Lys Gly Phe Asp
165 170 175

Pro Lys Met Gly Phe Thr Lys Glu Gly Phe Leu Lys Phe Glu Thr Lys
180 185 190

Asp Leu Asn Val Ile Ile Tyr Gly Asn Phe Lys Thr Arg Val Thr Arg
195 200 205

Lys Arg Asp Gly Lys Gly Ile Met Glu Asn Asn Glu Val Pro Ala Gly
210 215 220

Ser Leu Gly Asn Lys Cys Arg Gly Leu Met Phe Val Asp Arg Leu Tyr
225 230 235 240
Gly Thr Ala Ile Ala Ser Val Asn Glu Asn Tyr Arg Asn Asp Pro Asp
245 250 255
Arg Lys Glu Gly Phe Tyr Gly Ala Gly Glu Val Asn Cys Glu Phe Trp
260 265 270
Asp Ser Glu Gln Asn Arg Asn Lys Tyr Ile Leu Glu Arg Thr Gly Ile
275 280 285
Ala Met Thr Asn Tyr Asn Tyr Asp Asn Tyr Asn Tyr Asn Gln Ser Asp
290 295 300
Leu Ile Ala Pro Gly Tyr Pro Ser Asp Pro Asn Phe Tyr Ile Pro Met
305 310 315 320
Tyr Phe Ala Ala Pro Trp Val Val Val Lys Gly Cys Ser Gly Asn Ser
325 330 335
Asp Glu Gln Tyr Ser Tyr Gly Trp Phe Met Asp Asn Val Ser Gln Thr
340 345 350
Tyr Met Asn Thr Gly Gly Thr Ser Trp Asn Cys Gly Glu Glu Asn Leu
355 360 365
Ala Tyr Met Gly Ala Gln Cys Gly Pro Phe Asp Gln His Phe Val Tyr
370 375 380
Gly Asp Gly Asp Gly Leu Glu Asp Val Val Gln Ala Phe Ser Leu Leu
385 390 395 400
Gln Gly Lys Glu Phe Glu Asn Gln Val Leu Asn Lys Arg Ala Val Met
405 410 415
Pro Pro Lys Tyr Val Phe Gly Tyr Phe Gln Gly Val Phe Gly Ile Ala
420 425 430
Ser Leu Leu Arg Glu Gln Arg Pro Glu Gly Gly Asn Asn Ile Ser Val
435 440 445
Gln Glu Ile Val Glu Gly Tyr Gln Ser Asn Asn Phe Pro Leu Glu Gly
450 455 460
Leu Ala Val Asp Val Asp Met Gln Gln Asp Leu Arg Val Phe Thr Thr
465 470 475 480
Lys Ile Glu Phe Trp Thr Ala Asn Lys Val Gly Thr Gly Gly Asp Ser
485 490 495
Asn Asn Lys Ser Val Phe Glu Trp Ala His Asp Lys Gly Leu Val Cys
500 505 510
Gln Thr Asn Val Thr Cys Phe Leu Arg Asn Asp Asn Gly Gly Ala Asp
515 520 525
Tyr Glu Val Asn Gln Thr Leu Arg Glu Lys Gly Leu Tyr Thr Lys Asn
530 535 540
Asp Ser Leu Thr Asn Thr Asn Phe Gly Thr Thr Asn Asp Gly Pro Ser
545 550 555 560
Asp Ala Tyr Ile Gly His Leu Asp Tyr Gly Gly Gly Gly Asn Cys Asp
565 570 575
Ala Leu Phe Pro Asp Trp Gly Arg Pro Gly Val Ala Glu Trp Trp Gly
580 585 590
Asp Asn Tyr Ser Lys Leu Phe Lys Ile Gly Leu Asp Phe Val Trp Gln
595 600 605
Asp Met Thr Val Pro Ala Met Met Pro His Lys Val Gly Asp Ala Val
610 615 620
Asp Thr Arg Ser Pro Tyr Gly Trp Pro Asn Glu Asn Asp Pro Ser Asn
625 630 635 640
Gly Arg Tyr Asn Trp Lys Ser Tyr His Pro Gln Val Leu Val Thr Asp
645 650 655
Met Arg Tyr Glu Asn His Gly Arg Glu Pro Met Phe Thr Gln Arg Asn
660 665 670
Met His Ala Tyr Thr Leu Cys Glu Ser Thr Arg Lys Glu Gly Ile Val
675 680 685
Ala Asn Ala Asp Thr Leu Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser
690 695 700
Arg Gly Gly Tyr Ile Gly Asn Gln His Phe Gly Gly Met Trp Val Gly
705 710 715 720
Asp Asn Ser Ser Ser Gln Arg Tyr Leu Gln Met Met Ile Ala Asn Ile
725 730 735
Val Asn Met Asn Met Ser Cys Leu Pro Leu Val Gly Ser Asp Ile Gly
740 745 750

Gly Phe Thr-Ser Tyr Asp Gly Arg Asn Val Cys Pro Gly Asp Leu Met
755 760 765
Val Arg Phe Val Gln Ala Gly Cys Leu Leu Pro Trp Phe Arg Asn His
770 775 780
Tyr Gly Arg Leu Val Glu Gly Lys Gln Glu Gly Lys Tyr Tyr Gln Glu
785 790 795 800
Leu Tyr Met Tyr Lys Asp Glu Met Ala Thr Leu Arg Lys Phe Ile Glu
805 810 815
Phe Arg Tyr Arg Trp Gln Glu Val Leu Tyr Thr Ala Met Tyr Gln Asn
820 825 830
Ala Ala Phe Gly Lys Pro Ile Ile Lys Ala Ala Ser Met Tyr Asp Asn
835 840 845
Asp Arg Asn Val Arg Gly Ala Gln Asp Asp His Phe Leu Leu Gly Gly
850 855 860
His Asp Gly Tyr Arg Ile Leu Cys Ala Pro Val Val Trp Glu Asn Thr
865 870 875 880
Thr Ser Arg Asp Leu Tyr Leu Pro Val Leu Thr Lys Trp Tyr Lys Phe
885 890 895
Gly Pro Asp Tyr Asp Thr Lys Arg Leu Asp Ser Ala Leu Asp Gly Gly
900 905 910
Gln Met Ile Lys Asn Tyr Ser Val Pro Gln Ser Asp Ser Pro Ile Phe
915 920 925
Val Arg Glu Gly Ala Ile Leu Pro Thr Arg Tyr Thr Leu Asp Gly Ser
930 935 940
Asn Lys Ser Met Asn Thr Tyr Thr Asp Lys Asp Pro Leu Val Phe Glu
945 950 955 960
Val Phe Pro Leu Gly Asn Asn Arg Ala Asp Gly Met Cys Tyr Leu Asp
965 970 975
Asp Gly Gly Ile Thr Thr Asp Ala Glu Asp His Gly Lys Phe Ser Val
980 985 990
Ile Asn Val Glu Ala Leu Arg Lys Gly Val Thr Thr Thr Ile Lys Phe
995 1000 1005
Ala Tyr Asp Thr Tyr Gln Tyr Val Phe Asp Gly Pro Phe Tyr Val Arg
1010 1015 1020
Ile Arg Asn Leu Thr Thr Ala Ser Lys Ile Asn Val Ser Ser Gly Ala
1025 1030 1035 1040
Gly Glu Glu Asp Met Thr Pro Thr Ser Ala Asn Ser Arg Ala Ala Leu
1045 1050 1055
Phe Ser Asp Gly Gly Val Gly Glu Tyr Trp Ala Asp Asn Asp Thr Ser
1060 1065 1070
Ser Leu Trp Met Lys Leu Pro Asn Leu Val Leu Gln Asp Ala Val Ile
1075 1080 1085
Thr Ile Thr
1090

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1066 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Ala Gly Phe Ser Asp Pro Leu Asn Phe Cys Lys Ala Glu Asp Tyr
1 5 10 15
Tyr Ser Val Ala Leu Asp Trp Lys Gly Pro Gln Lys Ile Ile Gly Val
20 25 30
Asp Thr Thr Pro Pro Lys Ser Thr Lys Phe Pro Lys Asn Trp His Gly
35 40 45
Val Asn Leu Arg Phe Asp Asp Gly Thr Leu Gly Val Val Gln Phe Ile
50 55 60
Arg Pro Cys Val Trp Arg Val Arg Tyr Asp Pro Gly Phe Lys Thr Ser
65 70 75 80

Asp Glu Tyr-Gly Asp Glu Asn Thr Arg Thr Ile Val Gln Asp Tyr Met
85 90 95
Ser Thr Leu Ser Asn Lys Leu Asp Thr Tyr Arg Gly Leu Thr Trp Glu
100 105 110
Thr Lys Cys Glu Asp Ser Gly Asp Phe Phe Thr Phe Ser Ser Lys Val
115 120 125
Thr Ala Val Glu Lys Ser Glu Arg Thr Arg Asn Lys Val Gly Asp Gly
130 135 140
Leu Arg Ile His Leu Trp Lys Ser Pro Phe Arg Ile Gln Val Val Arg
145 150 155 160
Thr Leu Thr Pro Leu Lys Asp Pro Tyr Pro Ile Pro Asn Val Ala Ala
165 170 175
Ala Glu Ala Arg Val Ser Asp Lys Val Val Trp Gln Thr Ser Pro Lys
180 185 190
Thr Phe Arg Lys Asn Leu His Pro Gln His Lys Met Leu Lys Asp Thr
195 200 205
Val Leu Asp Ile Val Lys Pro Gly His Gly Glu Tyr Val Gly Trp Gly
210 215 220
Glu Met Gly Gly Ile Gln Phe Met Lys Glu Pro Thr Phe Met Asn Tyr
225 230 235 240
Phe Asn Phe Asp Asn Met Gln Tyr Gln Gln Val Tyr Ala Gln Gly Ala
245 250 255
Leu Asp Ser Arg Glu Pro Leu Tyr His Ser Asp Pro Phe Tyr Leu Asp
260 265 270
Val Asn Ser Asn Pro Glu His Lys Asn Ile Thr Ala Thr Phe Ile Asp
275 280 285
Asn Tyr Ser Gln Ile Ala Ile Asp Phe Gly Lys Thr Asn Ser Gly Tyr
290 295 300
Ile Lys Leu Gly Thr Arg Tyr Gly Gly Ile Asp Cys Tyr Gly Ile Ser
305 310 315 320
Ala Asp Thr Val Pro Glu Ile Val Arg Leu Tyr Thr Gly Leu Val Gly
325 330 335
Arg Ser Lys Leu Lys Pro Arg Tyr Ile Leu Gly Ala His Gln Ala Cys
340 345 350
Tyr Gly Tyr Gln Gln Glu Ser Asp Leu Tyr Ser Val Val Gln Gln Tyr
355 360 365
Arg Asp Cys Lys Phe Pro Leu Asp Gly Ile His Val Asp Val Asp Val
370 375 380
Gln Asp Gly Phe Arg Thr Phe Thr Thr Asn Pro His Thr Phe Pro Asn
385 390 395 400
Pro Lys Glu Met Phe Thr Asn Leu Arg Asn Asn Gly Ile Lys Cys Ser
405 410 415
Thr Asn Ile Thr Pro Val Ile Ser Ile Asn Asn Arg Glu Gly Gly Tyr
420 425 430
Ser Thr Leu Leu Glu Gly Val Asp Lys Lys Tyr Phe Ile Met Asp Asp
435 440 445
Arg Tyr Thr Glu Gly Thr Ser Gly Asn Ala Lys Asp Val Arg Tyr Met
450 455 460
Tyr Tyr Gly Gly Gly Asn Lys Val Glu Val Asp Pro Asn Asp Val Asn
465 470 475 480
Gly Arg Pro Asp Phe Lys Asp Asn Tyr Asp Phe Pro Ala Asn Phe Asn
485 490 495
Ser Lys Gln Tyr Pro Tyr His Gly Gly Val Ser Tyr Gly Tyr Gly Asn
500 505 510
Gly Ser Ala Gly Phe Tyr Pro Asp Leu Asn Arg Lys Glu Val Arg Ile
515 520 525
Trp Trp Gly Met Gln Tyr Lys Tyr Leu Phe Asp Met Gly Leu Glu Phe
530 535 540
Val Trp Gln Asp Met Thr Thr Pro Ala Ile His Thr Ser Tyr Gly Asp
545 550 555 560
Met Lys Gly Leu Pro Thr Arg Leu Leu Val Thr Ser Asp Ser Val Thr
565 570 575
Asn Ala Ser Glu Lys Lys Leu Ala Ile Glu Thr Trp Ala Leu Tyr Ser
580 585 590
Tyr Asn Leu His Lys Ala Thr Trp His Gly Leu Ser Arg Leu Glu Ser
595 600 605

```
Arg Lys Asn Lys Arg Asn Phe Ile Leu Gly Arg Gly Ser Tyr Ala Gly
    610                 615                 620
Ala Tyr Arg Phe Ala Gly Leu Trp Thr Gly Asp Asn Ala Ser Asn Trp
625                 630                 635                 640
Glu Phe Trp Lys Ile Ser Val Ser Gln Val Leu Ser Leu Gly Leu Asn
                645                 650                 655
Gly Val Cys Ile Ala Gly Ser Asp Thr Gly Gly Phe Glu Pro Tyr Arg
            660                 665                 670
Asp Ala Asn Gly Val Glu Glu Lys Tyr Cys Ser Pro Glu Leu Leu Ile
        675                 680                 685
Arg Trp Tyr Thr Gly Ser Phe Leu Leu Pro Trp Leu Arg Asn His Tyr
    690                 695                 700
Val Lys Lys Asp Arg Lys Trp Phe Gln Glu Pro Tyr Ser Tyr Pro Lys
705                 710                 715                 720
His Leu Glu Thr His Pro Glu Leu Ala Asp Gln Ala Trp Leu Tyr Lys
                725                 730                 735
Ser Val Leu Glu Ile Cys Arg Tyr Tyr Val Glu Leu Arg Tyr Ser Leu
            740                 745                 750
Ile Gln Leu Leu Tyr Asp Cys Met Phe Gln Asn Val Val Asp Gly Met
        755                 760                 765
Pro Ile Thr Arg Ser Met Leu Leu Thr Asp Thr Glu Asp Thr Thr Phe
    770                 775                 780
Phe Asn Glu Ser Gln Lys Phe Leu Asp Asn Gln Tyr Met Ala Gly Asp
785                 790                 795                 800
Asp Ile Leu Val Ala Pro Ile Leu His Ser Arg Lys Glu Ile Pro Gly
                805                 810                 815
Glu Asn Arg Asp Val Tyr Leu Pro Leu Tyr His Thr Trp Tyr Pro Ser
            820                 825                 830
Asn Leu Arg Pro Trp Asp Asp Gln Gly Val Ala Leu Gly Asn Pro Val
        835                 840                 845
Glu Gly Gly Ser Val Ile Asn Tyr Thr Ala Arg Ile Val Ala Pro Glu
    850                 855                 860
Asp Tyr Asn Leu Phe His Ser Val Val Pro Val Tyr Val Arg Glu Gly
865                 870                 875                 880
Ala Ile Ile Pro Gln Ile Glu Val Arg Gln Trp Thr Gly Gln Gly Gly
                885                 890                 895
Ala Asn Arg Ile Lys Phe Asn Ile Tyr Pro Gly Lys Asp Lys Glu Tyr
            900                 905                 910
Cys Thr Tyr Leu Asp Asp Gly Val Ser Arg Asp Ser Ala Pro Glu Asp
        915                 920                 925
Leu Pro Gln Tyr Lys Glu Thr His Glu Gln Ser Lys Val Glu Gly Ala
    930                 935                 940
Glu Ile Ala Lys Gln Ile Gly Lys Lys Thr Gly Tyr Asn Ile Ser Gly
945                 950                 955                 960
Thr Asp Pro Glu Ala Lys Gly Tyr His Arg Lys Val Ala Val Thr Gln
                965                 970                 975
Thr Ser Lys Asp Lys Thr Arg Thr Val Thr Ile Glu Pro Lys His Asn
            980                 985                 990
Gly Tyr Asp Pro Ser Lys Glu Val Gly Asp Tyr Tyr Thr Ile Ile Leu
        995                 1000                1005
Trp Tyr Ala Pro Gly Phe Asp Gly Ser Ile Val Asp Val Ser Lys Thr
    1010                1015                1020
Thr Val Asn Val Glu Gly Gly Val Glu His Gln Val Tyr Lys Asn Ser
1025                1030                1035                1040
Asp Leu His Thr Val Val Ile Asp Val Lys Glu Val Ile Gly Thr Thr
                1045                1050                1055
Lys Ser Val Lys Ile Thr Cys Thr Ala Ala
            1060                1065
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1070 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Gly Leu Ser Asp Pro Leu Asn Phe Cys Lys Ala Glu Asp Tyr
1               5                   10                  15
Tyr Ala Ala Ala Lys Gly Trp Ser Gly Pro Gln Lys Ile Ile Arg Tyr
            20                  25                  30
Asp Gln Thr Pro Pro Gln Gly Thr Lys Asp Pro Lys Ser Trp His Ala
        35                  40                  45
Val Asn Leu Pro Phe Asp Asp Gly Thr Met Cys Val Val Gln Phe Val
    50                  55                  60
Arg Pro Cys Val Trp Arg Val Arg Tyr Asp Pro Ser Val Lys Thr Ser
65                  70                  75                  80
Asp Glu Tyr Gly Asp Glu Asn Thr Arg Thr Ile Val Gln Asp Tyr Met
                85                  90                  95
Thr Thr Leu Val Gly Asn Leu Asp Ile Phe Arg Gly Leu Thr Trp Val
            100                 105                 110
Ser Thr Leu Glu Asp Ser Gly Glu Tyr Tyr Thr Phe Lys Ser Glu Val
        115                 120                 125
Thr Ala Val Asp Glu Thr Glu Arg Thr Arg Asn Lys Val Gly Asp Gly
    130                 135                 140
Leu Lys Ile Tyr Leu Trp Lys Asn Pro Phe Arg Ile Gln Val Val Arg
145                 150                 155                 160
Leu Leu Thr Pro Leu Val Asp Pro Phe Pro Ile Pro Asn Val Ala Asn
                165                 170                 175
Ala Thr Ala Arg Val Ala Asp Lys Val Val Trp Gln Thr Ser Pro Lys
            180                 185                 190
Thr Phe Arg Lys Asn Leu His Pro Gln His Lys Met Leu Lys Asp Thr
        195                 200                 205
Val Leu Asp Ile Ile Lys Pro Gly His Gly Glu Tyr Val Gly Trp Gly
    210                 215                 220
Glu Met Gly Gly Ile Glu Phe Met Lys Glu Pro Thr Phe Met Asn Tyr
225                 230                 235                 240
Phe Asn Phe Asp Asn Met Gln Tyr Gln Gln Val Tyr Ala Gln Gly Ala
                245                 250                 255
Leu Asp Ser Arg Glu Pro Leu Tyr His Ser Asp Pro Phe Tyr Leu Asp
            260                 265                 270
Val Asn Ser Asn Pro Glu His Lys Asn Ile Thr Ala Thr Phe Ile Asp
        275                 280                 285
Asn Tyr Ser Gln Ile Ala Ile Asp Phe Gly Lys Thr Asn Ser Gly Tyr
    290                 295                 300
Ile Lys Leu Gly Thr Arg Tyr Gly Gly Ile Asp Cys Tyr Gly Ile Ser
305                 310                 315                 320
Ala Asp Thr Val Pro Glu Ile Val Arg Leu Tyr Thr Gly Leu Val Gly
                325                 330                 335
Arg Ser Lys Leu Lys Pro Arg Tyr Ile Leu Gly Ala His Gln Ala Cys
            340                 345                 350
Tyr Gly Tyr Gln Gln Glu Ser Asp Leu His Ala Val Val Gln Gln Tyr
        355                 360                 365
Arg Asp Thr Lys Phe Pro Leu Asp Gly Leu His Val Asp Val Asp Phe
    370                 375                 380
Gln Asp Asn Phe Arg Thr Phe Thr Thr Asn Pro Ile Thr Phe Pro Asn
385                 390                 395                 400
Pro Lys Glu Met Phe Thr Asn Leu Arg Asn Asn Gly Ile Lys Cys Ser
                405                 410                 415
Thr Asn Ile Thr Pro Val Ile Ser Ile Arg Asp Arg Pro Asn Gly Tyr
            420                 425                 430
Ser Thr Leu Asn Glu Gly Tyr Asp Lys Lys Tyr Phe Ile Met Asp Asp
        435                 440                 445
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Arg Tyr Thr Glu Gly Thr Ser Gly Asp Pro Gln Asn Val Arg Tyr Ser
450 455 460

Phe Tyr Gly Gly Gly Asn Pro Val Glu Val Asn Pro Asn Asp Val Trp
465 470 475 480

Ala Arg Pro Asp Phe Gly Asp Asn Tyr Asp Phe Pro Thr Asn Phe Asn
485 490 495

Cys Lys Asp Tyr Pro Tyr His Gly Gly Val Ser Tyr Gly Tyr Gly Asn
500 505 510

Gly Thr Pro Gly Tyr Tyr Pro Asp Leu Asn Arg Glu Glu Val Arg Ile
515 520 525

Trp Trp Gly Leu Gln Tyr Glu Tyr Leu Phe Asn Met Gly Leu Glu Phe
530 535 540

Val Trp Gln Asp Met Thr Thr Pro Ala Ile His Ser Ser Tyr Gly Asp
545 550 555 560

Met Lys Gly Leu Pro Thr Arg Leu Leu Val Thr Ala Asp Ser Val Thr
565 570 575

Asn Ala Ser Glu Lys Lys Leu Ala Ile Glu Ser Trp Ala Leu Tyr Ser
580 585 590

Tyr Asn Leu His Lys Ala Thr Phe His Gly Leu Gly Arg Leu Glu Ser
595 600 605

Arg Lys Asn Lys Arg Asn Phe Ile Leu Gly Arg Gly Ser Tyr Ala Gly
610 615 620

Ala Tyr Arg Phe Ala Gly Leu Trp Thr Gly Asp Asn Ala Ser Thr Trp
625 630 635 640

Glu Phe Trp Lys Ile Ser Val Ser Gln Val Leu Ser Leu Gly Leu Asn
645 650 655

Gly Val Cys Ile Ala Gly Ser Asp Thr Gly Gly Phe Glu Pro Ala Arg
660 665 670

Thr Glu Ile Gly Glu Glu Lys Tyr Cys Ser Pro Glu Leu Leu Ile Arg
675 680 685

Trp Tyr Thr Gly Ser Phe Leu Leu Pro Trp Leu Arg Asn His Tyr Val
690 695 700

Lys Lys Asp Arg Lys Trp Phe Gln Glu Pro Tyr Ala Tyr Pro Lys His
705 710 715 720

Leu Glu Thr His Pro Glu Leu Ala Asp Gln Ala Trp Leu Tyr Lys Ser
725 730 735

Val Leu Glu Ile Cys Arg Tyr Trp Val Glu Leu Arg Tyr Ser Leu Ile
740 745 750

Gln Leu Leu Tyr Asp Cys Met Phe Gln Asn Val Val Asp Gly Met Pro
755 760 765

Leu Ala Arg Ser Met Leu Leu Thr Asp Thr Glu Asp Thr Thr Phe Phe
770 775 780

Asn Glu Ser Gln Lys Phe Leu Asp Asn Gln Tyr Met Ala Gly Asp Asp
785 790 795 800

Ile Leu Val Ala Pro Ile Leu His Ser Arg Asn Glu Val Pro Gly Glu
805 810 815

Asn Arg Asp Val Tyr Leu Pro Leu Phe His Thr Trp Tyr Pro Ser Asn
820 825 830

Leu Arg Pro Trp Asp Asp Gln Gly Val Ala Leu Gly Asn Pro Val Glu
835 840 845

Gly Gly Ser Val Ile Asn Tyr Thr Ala Arg Ile Val Ala Pro Glu Asp
850 855 860

Tyr Asn Leu Phe His Asn Val Val Pro Val Tyr Ile Arg Glu Gly Ala
865 870 875 880

Ile Ile Pro Gln Ile Gln Val Arg Gln Trp Ile Gly Glu Gly Gly Pro
885 890 895

Asn Pro Ile Lys Phe Asn Ile Tyr Pro Gly Lys Asp Lys Glu Tyr Val
900 905 910

Thr Tyr Leu Asp Asp Gly Val Ser Arg Asp Ser Ala Pro Asp Asp Leu
915 920 925

Pro Gln Tyr Arg Glu Ala Tyr Glu Gln Ala Lys Val Glu Gly Lys Asp
930 935 940

Val Gln Lys Gln Leu Ala Val Ile Gln Gly Asn Lys Thr Asn Asp Phe
945 950 955 960

Ser Ala Ser Gly Ile Asp Lys Glu Ala Lys Gly Tyr His Arg Lys Val
965 970 975

Ser Ile Lys Gln Glu Ser Lys Asp Lys Thr Arg Thr Val Thr Ile Glu
980 985 990
Pro Lys His Asn Gly Tyr Asp Pro Ser Lys Glu Val Gly Asn Tyr Tyr
995 1000 1005
Thr Ile Ile Leu Trp Tyr Ala Pro Gly Phe Asp Gly Ser Ile Val Asp
1010 1015 1020
Val Ser Gln Ala Thr Val Asn Ile Glu Gly Gly Val Glu Cys Glu Ile
1025 1030 1035 1040
Phe Lys Asn Thr Gly Leu His Thr Val Val Val Asn Val Lys Glu Val
1045 1050 1055
Ile Gly Thr Thr Lys Ser Val Lys Ile Thr Cys Thr Thr Ala
1060 1065 1070

SEQ. ID. NO. 5

SEQUENCE TYPE: ENZYME
MOLECULE TYPE: AMINO ACID
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 1092 AMINO ACIDS
SEQUENCE:

5 10 15

1 Met Phe Pro Thr Leu Thr Phe Ile Ala Pro Ser Ala Leu Ala Ala
16 Ser Thr Phe Val Gly Ala Asp Ile Arg Ser Gly Ile Arg Ile Gln
31 Ser Ala Leu Pro Ala Val Arg Asn Ala Val Arg Arg Ser Lys His
46 Tyr Asn Val Ser Met Thr Ala Leu Ser Asp Lys Gln Thr Ala Ile
61 Ser Ile Gly Pro Asp Asn Pro Asp Gly Ile Asn Tyr Gln Asn Tyr
76 Asp Tyr Ile Pro Val Ala Gly Phe Thr Pro Leu Ser Asn Thr Asn
91 Trp Tyr Ala Ala Gly Ser Ser Thr Pro Gly Gly Ile Thr Asp Trp
106 Thr Ala Thr Met Asn Val Lys Phe Asp Arg Ile Asp Asn Pro Ser
121 Tyr Ser Asn Asn His Pro Val Gln Ile Gln Val Thr Ser Tyr Asn
136 Asn Asn Ser Phe Arg Ile Arg Phe Asn Pro Asp Gly Pro Ile Arg
151 Asp Val Ser Arg Gly Pro Ile Leu Lys Gln Gln Leu Thr Trp Ile
166 Arg Asn Gln Glu Leu Ala Gln Gly Cys Asn Pro Asn Met Ser Phe
181 Ser Pro Glu Gly Phe Leu Ser Phe Glu Thr Lys Asp Leu Asn Val
196 Ile Ile Tyr Gly Asn Cys Lys Met Arg Val Thr Lys Lys Asp Gly
211 Tyr Leu Val Met Glu Asn Asp Glu Cys Asn Ser Gln Ser Asp Gly
226 Asn Lys Cys Arg Gly Leu Met Tyr Val Asp Arg Leu Tyr Gly Asn
241 Ala Ile Ala Ser Val Gln Thr Asn Phe His Lys Asp Thr Ser Arg
256 Asn Glu Lys Phe Tyr Gly Ala Gly Glu Val Asn Cys Arg Tyr Glu
271 Glu Gln Gly Lys Ala Pro Thr Tyr Val Leu Glu Arg Ser Gly Leu
286 Ala Met Thr Asn Tyr Asn Tyr Asp Asn Leu Asn Tyr Asn Gln Pro
301 Asp Val Val Pro Pro Gly Tyr Pro Asp His Pro Asn Tyr Tyr Ile
316 Pro Met Tyr Tyr Ala Ala Pro Trp Leu Val Val Gln Gly Cys Ala
331 Gly Thr Ser Lys Gln Tyr Ser Tyr Gly Trp Phe Met Asp Asn Val
346 Ser Gln Ser Tyr Met Asn Thr Gly Asp Thr Ala Trp Asn Cys Gly
361 Gln Glu Asn Leu Ala Tyr Met Gly Ala Gln Tyr Gly Pro Phe Asp
376 Gln His Phe Val Tyr Gly Asp Gly Asp Gly Leu Glu Asp Val Val
391 Lys Ala Phe Ser Phe Leu Gln Gly Lys Glu Phe Glu Asp Lys Lys
406 Leu Asn Lys Arg Ser Val Met Pro Pro Lys Tyr Val Phe Gly Phe
421 Phe Gln Gly Val Phe Gly Ala Leu Ser Leu Leu Lys Gln Asn Leu
436 Pro Ala Gly Glu Asn Asn Ile Ser Val Gln Glu Ile Val Glu Gly
451 Tyr Gln Asp Asn Asp Tyr Pro Phe Glu Gly Leu Ala Val Asp Val
466 Asp Met Gln Asp Asp Leu Arg Val Phe Thr Thr Lys Pro Glu Tyr
481 Trp Ser Ala Asn Met Val Gly Glu Gly Gly Asp Pro Asn Asn Arg
496 Ser Val Phe Glu Trp Ala His Asp Arg Gly Leu Val Cys Gln Thr
511 Asn Val Thr Cys Phe Leu Arg Asn Asp Asn Ser Gly Lys Pro Tyr
526 Glu Val Asn Gln Thr Leu Arg Glu Lys Gln Leu Tyr Thr Lys Asn
541 Asp Ser Leu Asn Asn Thr Asp Phe Gly Thr Thr Ser Asp Gly Pro
556 Gly Asp Ala Tyr Ile Gly His Leu Asp Tyr Gly Gly Gly Val Glu
571 Cys Asp Ala Ile Phe Pro Asp Trp Gly Arg Pro Asp Val Ala Gln
586 Trp Trp Gly Glu Asn Tyr Lys Lys Leu Phe Ser Ile Gly Leu Asp
601 Phe Val Trp Gln Asp Met Thr Val Pro Ala Met Met Pro His Arg
616 Leu Gly Asp Ala Val Asn Lys Asn Ser Gly Ser Ser Ala Pro Gly
631 Trp Pro Asn Glu Asn Asp Pro Ser Asn Gly Arg Tyr Asn Trp Lys
646 Ser Tyr His Pro Gln Val Leu Val Thr Asp Met Arg Tyr Gly Ala
661 Glu Tyr Gly Arg Glu Pro Met Val Ser Gln Arg Asn Ile His Ala

676 Tyr Thr Leu Cys Glu Ser Thr Arg-Arg Glu Gly Ile Val Gly Asn
691 Ala Asp Ser Leu Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg
706 Gly Gly Tyr Ile Gly Asn Gln His Phe Gly Gly Met Trp Val Gly
721 Asp Asn Ser Ala Thr Glu Ser Tyr Leu Gln Met Met Leu Ala Asn
736 Ile Ile Asn Met Asn Met Ser Cys Leu Pro Leu Val Gly Ser Asp
751 Ile Gly Gly Phe Thr Gln Tyr Asn Asp Ala Gly Asp Pro Thr Pro
766 Glu Asp Leu Met Val Arg Phe Val Gln Ala Gly Cys Leu Leu Pro
781 Trp Phe Arg Asn His Tyr Asp Arg Trp Ile Glu Ser Lys Lys His
796 Gly Lys Lys Tyr Gln Glu Leu Tyr Met Tyr Pro Gly Gln Lys Asp
811 Thr Leu Lys Lys Phe Val Glu Phe Arg Tyr Arg Trp Gln Glu Val
826 Leu Tyr Thr Ala Met Tyr Gln Asn Ala Thr Thr Gly Glu Pro Ile
841 Ile Lys Ala Ala Pro Met Tyr Asn Asn Asp Val Asn Val Tyr Lys
856 Ser Gln Asn Asp His Phe Leu Leu Gly Gly His Asp Gly Tyr Arg
871 Ile Leu Cys Ala Pro Val Val Arg Glu Asn Ala Thr Ser Arg Glu
886 Val Tyr Leu Pro Val Tyr Ser Lys Trp Phe Lys Phe Gly Pro Asp
901 Phe Asp Thr Lys Pro Leu Glu Asn Glu Ile Gln Gly Gly Gln Thr
916 Leu Tyr Asn Tyr Ala Ala Pro Leu Asn Asp Ser Pro Ile Phe Val
931 Arg Glu Gly Thr Ile Leu Pro Thr Arg Tyr Thr Leu Asp Gly Val
946 Asn Lys Ser Ile Asn Thr Tyr Thr Asp Asn Asp Pro Leu Val Phe
961 Glu Leu Phe Pro Leu Glu Asn Asn Gln Ala His Gly Leu Phe Tyr
976 His Asp Asp Gly Gly Val Thr Thr Asn Ala Glu Asp Phe Gly Lys
991 Tyr Ser Val Ile Ser Val Lys Ala Ala Gln Glu Gly Ser Gln Met
1006 Ser Val Lys Phe Asp Asn Glu Val Tyr Glu His Gln Trp Gly Ala
1021 Ser Phe Tyr Val Arg Val Arg Asn Met Gly Ala Pro Ser Asn Ile
1036 Asn Val Ser Ser Gln Ile Gly Gln Gln Asp Met Gln Gln Ser Ser
1051 Val Ser Ser Arg Ala Gln Met Phe Thr Ser Ala Asn Asp Gly Glu
1066 Tyr Trp Val Asp Gln Ser Thr Asn Ser Leu Trp Leu Lys Leu Pro
1081 Gly Ala Val Ile Gln Asp Ala Ala Ile Thr Val Arg

Number of amino acid residues: 1092

Amino acid composition (including the signal sequense):

| | | | | |
|---|---|---|---|---|
| 64 Ala | 14 Cys | 18 His | 33 Met | 56 Thr |
| 48 Arg | 55 Gln | 45 Ile | 49 Phe | 22 Trp |
| 89 Asn | 49 Glu | 65 Leu | 59 Pro | 67 Tyr |
| 73 Asp | 94 Gly | 46 Lys | 73 Ser | 73 Val |

SEQ. ID. NO. 6

SEQUENCE TYPE: ENZYME
MOLECULE TYPE: AMINO ACID
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 570 AMINO ACIDS
SEQUENCE:

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                       5                   10                  15
                       |                   |                   |
  1 Met Thr Asn Tyr Asn Tyr Asp Asn Leu Asn Tyr Asn Gln Pro Asp
 16 Leu Ile Pro Pro Gly His Asp Ser Asp Pro Asp Tyr Tyr Ile Pro
 31 Met Tyr Phe Ala Ala Pro Trp Val Ile Ala His Gly Tyr Arg Gly
 46 Thr Ser Asp Gln Tyr Ser Tyr Gly Trp Phe Leu Asp Asn Val Ser
 61 Gln Ser Tyr Thr Asn Thr Gly Asp Asp Ala Trp Ala Gly Gln Lys
 76 Asp Leu Ala Tyr Met Gly Ala Gln Cys Gly Pro Phe Asp Gln His
 91 Phe Val Tyr Glu Ala Gly Asp Gly Leu Glu Asp Val Val Thr Ala
106 Phe Ser Tyr Leu Gln Gly Lys Glu Tyr Glu Asn Gln Gly Leu Asn
121 Ile Arg Ser Ala Met Pro Pro Lys Tyr Val Phe Gly Phe Phe Gln
136 Gly Val Phe Gly Ala Thr Ser Leu Leu Arg Asp Asn Leu Pro Ala
151 Gly Glu Asn Asn Val Ser Leu Glu Glu Ile Val Glu Gly Tyr Gln
166 Asn Gln Asn Val Pro Phe Glu Gly Leu Ala Val Asp Val Asp Met
181 Gln Asp Asp Leu Arg Val Phe Thr Thr Arg Pro Ala Phe Trp Thr
196 Ala Asn Lys Val Gly Glu Gly Gly Asp Pro Asn Asn Lys Ser Val
211 Phe Glu Trp Ala His Asp Arg Gly Leu Val Cys Gln Thr Asn Val
226 Thr Cys Phe Leu Lys Asn Glu Lys Asn Pro Tyr Glu Val Asn Gln
241 Ser Leu Arg Glu Lys Gln Leu Tyr Thr Lys Ser Asp Ser Leu Asp
256 Asn Ile Asp Phe Gly Thr Thr Pro Asp Gly Pro Ser Asp Ala Tyr
271 Ile Gly His Leu Asp Tyr Gly Gly Gly Val Glu Cys Asp Ala Leu
286 Phe Pro Asp Trp Gly Arg Pro Asp Val Ala Gln Trp Trp Gly Asp
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301 Asn Tyr Lys Lys Leu Phe Ser Ile Gly Leu Asp Phe Val Trp Gln
316 Asp Met Thr Val Pro Ala Met Met Pro His Arg Leu Gly Asp Pro
331 Val Gly Thr Asn Ser Gly Glu Thr Ala Pro Gly Trp Pro Asn Asp
346 Lys Asp Pro Ser Asn Gly Arg Tyr Asn Trp Lys Ser Tyr His Pro
361 Gln Val Leu Val Thr Asp Met Arg Tyr Asp Asp Tyr Gly Arg Asp
376 Pro Ile Val Thr Gln Arg Asn Leu His Ala Tyr Thr Leu Cys Glu
391 Ser Thr Arg Arg Glu Gly Ile Val Gly Asn Ala Asp Ser Leu Thr
406 Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg Gly Gly Tyr Ile Gly
421 Asn Gln His Phe Gly Gly Met Trp Val Gly Asp Asn Ser Ser Thr
436 Glu Asp Tyr Leu Ala Met Met Val Ile Asn Val Ile Asn Met Asn
451 Met Ser Gly Val Pro Leu Val Gly Ser Asp Ile Gly Gly Phe Thr
466 Glu His Asp Lys Arg Asn Pro Cys Thr Pro Asp Leu Met Met Arg
481 Phe Val Gln Ala Gly Cys Leu Leu Pro Trp Phe Arg Asn His Tyr
496 Asp Arg Trp Ile Glu Ser Lys Lys His Gly Lys Asn Tyr Gln Glu
511 Leu Tyr Met Tyr Arg Asp His Leu Asp Ala Leu Arg Ser Phe Val
526 Glu Leu Arg Tyr Arg Trp Gln Glu Val Leu Tyr Thr Ala Met Tyr
541 Gln Asn Ala Leu Asn Gly Lys Pro Ile Ile Lys Thr Val Ser Met
556 Tyr Asn Asn Asp Met Asn Val Lys Asp Ala Gln Asn Asp His Phe

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATGTTTTCAA CCCTTGCGTT TGTCGCACCT AGTGCGCTGG GAGCCAGTAC CTTCGTAGGG 60
GCGGAGGTCA GGTCAAATGT TCGTATCCAT TCCGCTTTTC CAGCTGTGCA CACAGCTACT 120
CGCAAAACCA ATCGCCTCAA TGTATCCATG ACCGCATTGT CCGACAAACA AACGGCTACT 180
GCGGGTAGTA CAGACAATCC GGACGGTATC GACTACAAGA CCTACGATTA CGTCGGAGTA 240
TGGGGTTTCA GCCCCCTCTC CAACACGAAC TGGTTTGCTG CCGGCTCTTC TACCCCGGGT 300
GGCATCACTG ATTGGACGGC TACAATGAAT GTCAACTTCG ACCGTATCGA CAATCCGTCC 360
ATCACTGTCC AGCATCCCGT TCAGGTTCAG GTCACGTCAT ACAACAACAA CAGCTACAGG 420
GTTCGCTTCA ACCCTGATGG CCCTATTCGT GATGTGACTC GTGGGCCTAT CCTCAAGCAG 480
CAACTAGATT GGATTCGAAC GCAGGAGCTG TCAGAGGGAT GTGATCCCGG AATGACTTTC 540
ACATCAGAAG GTTTCTTGAC TTTTGAGACC AAGGATCTAA GCGTCATCAT CTACGGAAAT 600
TTCAAGACCA GAGTTACGAG AAAGTCTGAC GGCAAGGTCA TCATGGAAAA TGATGAAGTT 660
GGAACTGCAT CGTCCGGGAA CAAGTGCCGG GGATTGATGT TCGTTGATAG ATTATACGGT 720
AACGCTATCG CTTCCGTCAA CAAGAACTTC CGCAACGACG CGGTCAAGCA GGAGGGATTC 780
TATGGTGCAG GTGAAGTCAA CTGTAAGTAC CAGGACACCT ACATCTTAGA ACGCACTGGA 840
ATCGCCATGA CAAATTACAA CTACGATAAC TTGAACTATA ACCAGTGGGA CCTTAGACCT 900
CCGCATCATG ATGGTGCCCT CAACCCAGAC TATTATATTC CAATGTACTA CGCAGCACCT 960
TGGTTGATCG TTAATGGATG CGCCGGTACT TCGGAGCAGT ACTCGTATGG ATGGTTCATG 1020
GACAATGTCT CTCAATCTTA CATGAATACT GGAGATACTA CCTGGAATTC TGGACAAGAG 1080
GACCTGGCAT ACATGGGCGC GCAGTATGGA CCATTTGACC AACATTTTGT TTACGGTGCT 1140
GGGGGTGGGA TGGAATGTGT GGTCACAGCG TTCTCTCTTC TACAAGGCAA GGAGTTCGAG 1200
AACCAAGTTC TCAACAAACG TTCAGTAATG CCTCCGAAAT ACGTCTTTGG TTTCTTCCAG 1260
GGTGTTTTCG GGACTTCTTC CTTGTTGAGA GCGCATATGC CAGCAGGTGA GAACAACATC 1320
TCAGTCGAAG AAATTGTAGA AGGTTATCAA AACAACAATT TCCCTTTCGA GGGGCTCGCT 1380
GTGGACGTGG ATATGCAAGA CAACTTGCGG GTGTTCACCA CGAAGGGCGA ATTTTGGACC 1440
GCAAACAGGG TGGGTACTGG CGGGGATCCA AACAACCGAT CGGTTTTTGA ATGGGCACAT 1500
GACAAAGGCC TTGTTTGTCA GACAAATATA ACTTGCTTCC TGAGGAATGA TAACGAGGGG 1560
CAAGACTACG AGGTCAATCA GACGTTAAGG GAGAGGCAGT TGTACACGAA GAACGACTCC 1620
CTGACGGGTA CGGATTTTGG AATGACCGAC GACGGCCCCA GCGATGCGTA CATCGGTCAT 1680
CTGGACTATG GGGGTGGAGT AGAATGTGAT GCACTTTTCC CAGACTGGGG ACGGCCTGAC 1740
GTGGCCGAAT GGTGGGGAAA TAACTATAAG AAACTGTTCA GCATTGGTCT CGACTTCGTC 1800
TGGCAAGACA TGACTGTTCC AGCAATGATG CCGCACAAAA TTGGCGATGA CATCAATGTG 1860
AAACCGGATG GGAATTGGCC GAATGCGGAC GATCCGTCCA ATGGACAATA CAACTGGAAG 1920
ACGTACCATC CCCAAGTGCT TGTAACTGAT ATGCGTTATG AGAATCATGG TCGGGAACCG 1980
ATGGTCACTC AACGCAACAT TCATGCGTAT ACACTGTGCG AGTCTACTAG GAAGGAAGGG 2040
ATCGTGGAAA ACGCAGACAC TCTAACGAAG TTCCGCCGTA GCTACATTAT CAGTCGTGGT 2100
GGTTACATTG GTAACCAGCA TTTCGGGGGT ATGTGGGTGG GAGACAACTC TACTACATCA 2160

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AACTACATCC AAATGATGAT TGCCAACAAT ATTAACATGA ATATGTCTTG CTTGCCTCTC   2220
GTCGGCTCCG ACATTGGAGG ATTCACCTCA TACGACAATG AGAATCAGCG AACGCCGTGT   2280
ACCGGGGACT TGATGGTGAG GTATGTGCAG GCGGGCTGCC TGTTGCCGTG GTTCAGGAAC   2340
CACTATGATA GGTGGATCGA GTCCAAGGAC CACGGAAAGG ACTACCAGGA GCTGTACATG   2400
TATCCGAATG AAATGGATAC GTTGAGGAAG TTCGTTGAAT TCCGTTATCG CTGGCAGGAA   2460
GTGTTGTACA CGGCCATGTA CCAGAATGCG GCTTTCGGAA AGCCGATTAT CAAGGCTGCT   2520
TCGATGTACA ATAACGACTC AAACGTTCGC AGGGCGCAGA ACGATCATTT CCTTCTTGGT   2580
GGACATGATG GATATCGCAT TCTGTGCGCG CCTGTTGTGT GGGAGAATTC GACCGAACGC   2640
GAATTGTACT TGCCCGTGCT GACCCAATGG TACAAATTCG GTCCCGACTT TGACACCAAG   2700
CCTCTGGAAG GAGCGATGAA CGGAGGGGAC CGAATTTACA ACTACCCTGT ACCGCAAAGT   2760
GAATCACCAA TCTTCGTGAG AGAAGGTGCG ATTCTCCCTA CCCGCTACAC GTTGAACGGT   2820
GAAAACAAAT CATTGAACAC GTACACGGAC GAAGATCCGT TGGTGTTTGA AGTATTCCCC   2880
CTCGGAAACA ACCGTGCCGA CGGTATGTGT TATCTTGATG ATGGCGGTGT GACCACCAAT   2940
GCTGAAGACA ATGGCAAGTT CTCTGTCGTC AAGGTGGCAG CGGAGCAGGA TGGTGGTACG   3000
GAGACGATAA CGTTTACGAA TGATTGCTAT GAGTACGTTT TCGGTGGACC GTTCTACGTT   3060
CGAGTGCGCG GCGCTCAGTC GCCGTCGAAC ATCCACGTGT CTTCTGGAGC GGGTTCTCAG   3120
GACATGAAGG TGAGCTCTGC CACTTCCAGG GCTGCGCTGT TCAATGACGG GGAGAACGGT   3180
GATTTCTGGG TTGACCAGGA GACAGATTCT CTGTGGCTGA AGTTGCCCAA CGTTGTTCTC   3240
CCGGACGCTG TGATCACAAT TACCTAA                                       3267
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(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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ATGTATCCAA CCCTCACCTT CGTGGCGCCT AGTGCGCTAG GGGCCAGAAC TTTCACGTGT     60
GTGGGCATTT TTAGGTCACA CATTCTTATT CATTCGGTTG TTCCAGCGGT GCGTCTAGCT    120
GTGCGCAAAA GCAACCGCCT CAATGTATCC ATGTCCGCTT TGTTCGACAA ACCGACTGCT    180
GTTACTGGAG GGAAGGACAA CCCGGACAAT ATCAATTACA CCACTTATGA CTACGTCCCT    240
GTGTGGCGCT TCGACCCCCT CAGCAATACG AACTGGTTTG CTGCCGGATC TTCCACTCCC    300
GGCGATATTG ACGACTGGAC GGCGACAATG AATGTGAACT TCGACCGTAT CGACAATCCA    360
TCCTTCACTC TCGAGAAACC GGTTCAGGTT CAGGTCACGT CATACAAGAA CAATTGTTTC    420
AGGGTTCGCT TCAACCCTGA TGGTCCTATT CGCGATGTGG ATCGTGGGCC TATCCTCCAG    480
CAGCAACTAA ATTGGATCCG GAAGCAGGAG CAGTCGAAGG GGTTTGATCC TAAGATGGGC    540
TTCACAAAAG AAGGTTTCTT GAAATTTGAG ACCAAGGATC TGAACGTTAT CATATATGGC    600
AATTTTAAGA CTAGAGTTAC GAGGAAGAGG GATGGAAAAG GGATCATGGA GAATAATGAA    660
GTGCCGGCAG GATCGTTAGG GAACAAGTGC CGGGGATTGA TGTTTGTCGA CAGGTTGTAC    720
GGCACTGCCA TCGCTTCCGT TAATGAAAAT TACCGCAACG ATCCCGACAG GAAAGAGGGG    780
TTCTATGGTG CAGGAGAAGT AAACTGCGAG TTTTGGGACT CCGAACAAAA CAGGAACAAG    840
TACATCTTAG AACGAACTGG AATCGCCATG ACAAATTACA ATTATGACAA CTATAACTAC    900
AACCAGTCAG ATCTTATTGC TCCAGGATAT CCTTCCGACC CGAACTTCTA CATTCCCATG    960
TATTTTGCAG CACCTTGGGT AGTTGTTAAG GGATGCAGTG GCAACAGCGA TGAACAGTAC   1020
TCGTACGGAT GGTTTATGGA TAATGTCTCC CAAACTTACA TGAATACTGG TGGTACTTCC   1080
TGGAACTGTG GAGAGGAGAA CTTGGCATAC ATGGGAGCAC AGTGCGGTCC ATTTGACCAA   1140
CATTTTGTGT ATGGTGATGG AGATGGTCTT GAGGATGTTG TCCAAGCGTT CTCTCTTCTG   1200
CAAGGCAAAG AGTTTGAGAA CCAAGTTCTG AACAAACGTG CCGTAATGCC TCCGAAATAT   1260
GTGTTTGGTT ACTTTCAGGG AGTCTTTGGG ATTGCTTCCT TGTTGAGAGA GCAAAGACCA   1320
GAGGGTGGTA ATAACATCTC TGTTCAAGAG ATTGTCGAAG GTTACCAAAG CAATAACTTC   1380
CCTTTAGAGG GGTTAGCCGT AGATGTGGAT ATGCAACAAG ATTTGCGCGT GTTCACCACG   1440
AAGATTGAAT TTTGGACGGC AAATAAGGTA GGCACCGGGG GAGACTCGAA TAACAAGTCG   1500
GTGTTTGAAT GGGCACATGA CAAAGGCCTT GTATGTCAGA CGAATGTTAC TTGCTTCTTG   1560
AGAAACGACA ACGGCGGGGC AGATTACGAA GTCAATCAGA CATTGAGGGA GAAGGGTTTG   1620
TACACGAAGA ATGACTCACT GACGAACACT AACTTCGGAA CTACCAACGA CGGGCCGAGC   1680
GATGCGTACA TTGGACATCT GGACTATGGT GGCGGAGGGA ATTGTGATGC ACTTTTCCCA   1740
GACTGGGGTC GACCGGGTGT GGCTGAATGG TGGGGTGATA ACTACAGCAA GCTCTTCAAA   1800
ATTGGTCTGG ATTTCGTCTG GCAAGACATG ACAGTTCCAG CTATGATGCC ACACAAAGTT   1860
GGCGACGCAG TCGATACGAG ATCACCTTAC GGCTGGCCGA ATGAGAATGA TCCTTCGAAC   1920
GGACGATACA ATTGGAAATC TTACCATCCA CAAGTTCTCG TAACTGATAT GCGATATGAG   1980
AATCATGGAA GGGAACCGAT GTTCACTCAA CGCAATATGC ATGCGTACAC ACTCTGTGAA   2040
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TCTACGAGGA AGGAAGGGAT TGTTGCAAAT GCAGACACTC TAACGAAGTT CCGCCGCAGT 2100
TATATTATCA GTCGTGGAGG TTACATTGGC AACCAGCATT TTGGAGGAAT GTGGGTTGGA 2160
GACAACTCTT CCTCCCAAAG ATACCTCCAA ATGATGATCG CGAACATCGT CAACATGAAC 2220
ATGTCTTGCC TTCCACTAGT TGGGTCCGAC ATTGGAGGTT TTACTTCGTA TGATGGACGA 2280
AACGTGTGTC CCGGGGATCT AATGGTAAGA TTCGTGCAGG CGGGTTGCTT ACTACCGTGG 2340
TTCAGAAACC ACTATGGTAG GTTGGTCGAG GGCAAGCAAG AGGGAAAATA CTATCAAGAA 2400
CTGTACATGT ACAAGGACGA GATGGCTACA TTGAGAAAAT TCATTGAATT CCGTTACCGC 2460
TGGCAGGAGG TGTTGTACAC TGCTATGTAC CAGAATGCGG CTTTCGGGAA ACCGATTATC 2520
AAGGCAGCTT CCATGTACGA CAACGACAGA AACGTTCGCG GCGCACAGGA TGACCACTTC 2580
CTTCTCGGCG GACACGATGG ATATCGTATT TTGTGTGCAC CTGTTGTGTG GGAGAATACA 2640
ACCAGTCGCG ATCTGTACTT GCCTGTGCTG ACCAAATGGT ACAAATTCGG CCCTGACTAT 2700
GACACCAAGC GCCTGGATTC TGCGTTGGAT GGAGGGCAGA TGATTAAGAA CTATTCTGTG 2760
CCACAAAGCG ACTCTCCGAT ATTTGTGAGG GAAGGAGCTA TTCTCCCTAC CCGCTACACG 2820
TTGGACGGTT CGAACAAGTC AATGAACACG TACACAGACA AAGACCCGTT GGTGTTTGAG 2880
GTATTCCCTC TTGGAAACAA CCGTGCCGAC GGTATGTGTT ATCTTGATGA TGGCGGTATT 2940
ACTACAGATG CTGAGGACCA TGGCAAATTC TCTGTTATCA ATGTCGAAGC CTTACGGAAA 3000
GGTGTTACGA CGACGATCAA GTTTGCGTAT GACACTTATC AATACGTATT TGATGGTCCA 3060
TTCTACGTTC GAATCCGTAA TCTTACGACT GCATCAAAAA TTAACGTGTC TTCTGGAGCG 3120
GGTGAAGAGG ACATGACACC GACCTCTGCG AACTCGAGGG CAGCTTTGTT CAGTGATGGA 3180
GGTGTTGGAG AATACTGGGC TGACAATGAT ACGTCTTCTC TGTGGATGAA GTTGCCAAAC 3240
CTGGTTCTGC AAGACGCTGT GATTACCATT ACGTAG 3276

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3201 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ATGGCAGGAT TTTCTGATCC TCTCAACTTT TGCAAAGCAG AAGACTACTA CAGTGTTGCG 60
CTAGACTGGA AGGGCCCTCA AAAAATCATT GGAGTAGACA CTACTCCTCC AAAGAGCACC 120
AAGTTCCCCA AAAACTGGCA TGGAGTGAAC TTGAGATTCG ATGATGGGAC TTTAGGTGTG 180
GTTCAGTTCA TTAGGCCGTG CGTTTGGAGG GTTAGATACG ACCCTGGTTT CAAGACCTCT 240
GACGAGTATG GTGATGAGAA TACGAGGACA ATTGTGCAAG ATTATATGAG TACTCTGAGT 300
AATAAATTGG ATACTTATAG AGGTCTTACG TGGGAAACCA AGTGTGAGGA TTCGGGAGAT 360
TTCTTTACCT TCTCATCCAA GGTCACCGCC GTTGAAAAAT CCGAGCGGAC CCGCAACAAG 420
GTCGGCGATG GCCTCAGAAT TCACCTATGG AAAAGCCCTT TCCGCATCCA AGTAGTGCGC 480
ACCTTGACCC CTTTGAAGGA TCCTTACCCC ATTCCAAATG TAGCCGCAGC CGAAGCCCGT 540
GTGTCCGACA AGGTCGTTTG GCAAACGTCT CCCAAGACAT TCAGAAAGAA CCTGCATCCG 600
CAACACAAGA TGCTAAAGGA TACAGTTCTT GACATTGTCA AACCTGGACA TGGCGAGTAT 660
GTGGGGTGGG GAGAGATGGG AGGTATCCAG TTTATGAAGG AGCCAACATT CATGAACTAT 720
TTTAACTTCG ACAATATGCA ATACCAGCAA GTCTATGCCC AAGGTGCTCT CGATTCTCGC 780
GAGCCACTGT ACCACTCGGA TCCCTTCTAT CTTGATGTGA ACTCCAACCC GGAGCACAAG 840
AATATCACGG CAACCTTTAT CGATAACTAC TCTCAAATTG CCATCGACTT TGGAAAGACC 900
AACTCAGGCT ACATCAAGCT GGGAACCAGG TATGGTGGTA TCGATTGTTA CGGTATCAGT 960
GCGGATACGG TCCCGGAAAT TGTACGACTT TATACAGGTC TTGTTGGACG TTCAAAGTTG 1020
AAGCCCAGAT ATATTCTCGG GGCCCATCAA GCCTGTTATG GATACCAACA GGAAAGTGAC 1080
TTGTATTCTG TGGTCCAGCA GTACCGTGAC TGTAAATTTC CACTTGACGG GATTCACGTC 1140
GATGTCGATG TTCAGGACGG CTTCAGAACT TTCACCACCA ACCCACACAC TTTCCCTAAC 1200
CCCAAAGAGA TGTTTACTAA CTTGAGGAAT AATGGAATCA AGTGCTCCAC CAATATCACT 1260
CCTGTTATCA GCATTAACAA CAGAGAGGGT GGATACAGTA CCCTCCTTGA GGGAGTTGAC 1320
AAAAAATACT TTATCATGGA CGACAGATAT ACCGAGGGAA CAAGTGGGAA TGCGAAGGAT 1380
GTTCGGTACA TGTACTACGG TGGTGGTAAT AAGGTTGAGG TCGATCCTAA TGATGTTAAT 1440
GGTCGGCCAG ACTTTAAAGA CAACTATGAC TTCCCCGCGA ACTTCAACAG CAAACAATAC 1500
CCCTATCATG GTGGTGTGAG CTACGGTTAT GGGAACGGTA GTGCAGGTTT TTACCCGGAC 1560
CTCAACAGAA AGGAGGTTCG TATCTGGTGG GGAATGCAGT ACAAGTATCT CTTCGATATG 1620
GGACTGGAAT TTGTGTGGCA AGACATGACT ACCCCAGCAA TCCACACATC ATATGGAGAC 1680
ATGAAAGGGT TGCCCACCCG TCTACTCGTC ACCTCAGACT CCGTCACCAA TGCCTCTGAG 1740
AAAAAGCTCG CAATTGAAAC TTGGGCTCTC TACTCCTACA ATCTCCACAA AGCAACTTGG 1800
CATGGTCTTA GTCGTCTCGA ATCTCGTAAG AACAAACGAA ACTTCATCCT CGGGCGTGGA 1860
AGTTATGCCG GAGCCTATCG TTTTGCTGGT CTCTGGACTG GGGATAATGC AAGTAACTGG 1920
GAATTCTGGA AGATATCGGT CTCTCAAGTT CTTTCTCTGG GCCTCAATGG TGTGTGCATC 1980

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GCGGGGTCTG ATACGGGTGG TTTTGAACCC TACCGTGATG CAAATGGGGT CGAGGAGAAA   2040
TACTGTAGCC CAGAGCTACT CATCAGGTGG TATACTGGTT CATTCCTCTT GCCGTGGCTC   2100
AGGAACCATT ATGTCAAAAA GGACAGGAAA TGGTTCCAGG AACCATACTC GTACCCCAAG   2160
CATCTTGAAA CCCATCCAGA ACTCGCAGAC CAAGCATGGC TCTATAAATC CGTTTTGGAG   2220
ATCTGTAGGT ACTATGTGGA GCTTAGATAC TCCCTCATCC AACTACTTTA CGACTGCATG   2280
TTTCAAAACG TAGTCGACGG TATGCCAATC ACCAGATCTA TGCTCTTGAC CGATACTGAG   2340
GATACCACCT TCTTCAACGA GAGCCAAAAG TTCCTCGACA ACCAATATAT GGCTGGTGAC   2400
GACATTCTTG TTGCACCCAT CCTCCACAGT CGCAAAGAAA TTCCAGGCGA AAACAGAGAT   2460
GTCTATCTCC CTCTTTACCA CACCTGGTAC CCCTCAAATT TGAGACCATG GGACGATCAA   2520
GGAGTCGCTT TGGGGAATCC TGTCGAAGGT GGTAGTGTCA TCAATTATAC TGCTAGGATT   2580
GTTGCACCCG AGGATTATAA TCTCTTCCAC AGCGTGGTAC CAGTCTACGT TAGAGAGGGT   2640
GCCATCATCC CGCAAATCGA AGTACGCCAA TGGACTGGCC AGGGGGGAGC CAACCGCATC   2700
AAGTTCAACA TCTACCCTGG AAAGGATAAG GAGTACTGTA CCTATCTTGA TGATGGTGTT   2760
AGCCGTGATA GTGCGCCGGA AGACCTCCCA CAGTACAAAG AGACCCACGA ACAGTCGAAG   2820
GTTGAAGGCG CGGAAATCGC AAAGCAGATT GGAAAGAAGA CGGGTTACAA CATCTCAGGA   2880
ACCGACCCAG AAGCAAAGGG TTATCACCGC AAAGTTGCTG TCACACAAAC GTCAAAAGAC   2940
AAGACGCGTA CTGTCACTAT TGAGCCAAAA CACAATGGAT ACGACCCTTC CAAAGAGGTG   3000
GGTGATTATT ATACCATCAT TCTTTGGTAC GCACCAGGTT TCGATGGCAG CATCGTCGAT   3060
GTGAGCAAGA CGACTGTGAA TGTTGAGGGT GGGGTGGAGC ACCAAGTTTA TAAGAACTCC   3120
GATTTACATA CGGTTGTTAT CGACGTGAAG GAGGTGATCG GTACCACAAA GAGCGTCAAG   3180
ATCACATGTA CTGCCGCTTA A                                             3201
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3213 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS. double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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ATGGCAGGAT TATCCGACCC TCTCAATTTC TGCAAAGCAG AGGACTACTA CGCTGCTGCC     60
AAAGGCTGGA GTGGCCCTCA GAAGATCATT CGCTATGACC AGACCCCTCC TCAGGGTACA    120
AAAGATCCGA AAAGCTGGCA TGCGGTAAAC CTTCCTTTCG ATGACGGGAC TATGTGTGTA    180
GTGCAATTCG TCAGACCCTG TGTTTGGAGG GTTAGATATG ACCCCAGTGT CAAGACTTCT    240
GATGAGTACG GCGATGAGAA TACGAGGACT ATTGTACAAG ACTACATGAC TACTCTGGTT    300
GGAAACTTGG ACATTTTCAG AGGTCTTACG TGGGTTTCTA CGTTGGAGGA TTCGGGCGAG    360
TACTACACCT TCAAGTCCGA AGTCACTGCC GTGGACGAAA CCGAACGGAC TCGAAACAAG    420
GTCGGCGACG GCCTCAAGAT TTACCTATGG AAAAATCCCT TTCGCATCCA GGTAGTGCGT    480
CTCTTGACCC CCCTGGTGGA CCCTTTCCCC ATTCCCAACG TAGCCAATGC CACAGCCCGT    540
GTGGCCGACA AGGTTGTTTG GCAGACGTCC CCGAAGACGT TCAGGAAAAA CTTGCATCCG    600
CAGCATAAGA TGTTGAAGGA TACAGTTCTT GATATTATCA AGCCGGGGCA CGGAGAGTAT    660
GTGGGTTGGG GAGAGATGGG AGGCATCGAG TTTATGAAGG AGCCAACATT CATGAATTAT    720
TTCAACTTTG ACAATATGCA ATATCAGCAG GTCTATGCAC AAGGCGCTCT TGATAGTCGT    780
GAGCCGTTGT ATCACTCTGA TCCCTTCTAT CTCGACGTGA ACTCCAACCC AGAGCACAAG    840
AACATTACGG CAACCTTTAT CGATAACTAC TCTCAGATTG CCATCGACTT TGGGAAGACC    900
AACTCAGGCT ACATCAAGCT GGGTACCAGG TATGGCGGTA TCGATTGTTA CGGTATCAGC    960
GCGGATACGG TCCCGGAGAT TGTGCGACTT TATACTGGAC TTGTTGGGCG TTCGAAGTTG   1020
AAGCCCAGGT ATATTCTCGG AGCCCACCAA GCTTGTTATG GATACCAGCA GGAAAGTGAC   1080
TTGCATGCTG TTGTTCAGCA GTACCGTGAC ACCAAGTTTC CGCTTGATGG GTTGCATGTC   1140
GATGTCGACT TTCAGGACAA TTTCAGAACG TTTACCACTA ACCCGATTAC GTTCCCTAAT   1200
CCCAAAGAAA TGTTTACCAA TCTAAGGAAC AATGGAATCA AGTGTTCCAC CAACATCACC   1260
CCTGTTATCA GTATCAGAGA TCGCCCGAAT GGGTACAGTA CCCTCAATGA GGGATATGAT   1320
AAAAAGTACT TCATCATGGA TGACAGATAT ACCGAGGGGA CAAGTGGGGA CCCGCAAAAT   1380
GTTCGATACT CTTTTTACGG CGGTGGGAAC CCGGTTGAGG TTAACCCTAA TGATGTTTGG   1440
GCTCGGCCAG ACTTTGGAGA CAATTATGAC TTCCCTACGA ACTTCAACTG CAAAGACTAC   1500
CCCTATCATG GTGGTGTGAG TTACGGATAT GGGAATGGCA CTCCAGGTTA CTACCCTGAC   1560
CTTAACAGAG AGGAGGTTCG TATCTGGTGG GGATTGCAGT ACGAGTATCT CTTCAATATG   1620
GGACTAGAGT TTGTATGGCA AGATATGACA ACCCCAGCGA TCCATTCATC ATATGGAGAC   1680
ATGAAAGGGT TGCCCACCCG TCTGCTCGTC ACCGCCGACT CAGTTACCAA TGCCTCTGAG   1740
AAAAAGCTCG CAATTGAAAG TTGGGCTCTT TACTCCTACA ACCTCCATAA AGCAACCTTC   1800
CACGGTCTTG GTCGTCTTGA GTCTCGTAAG AACAAACGTA ACTTCATCCT CGGACGTGGT   1860
AGTTACGCCG GTGCCTATCG TTTTGCTGGT CTCTGGACTG GAGATAACGC AAGTACGTGG   1920
GAATTCTGGA AGATTTCGGT CTCCCAAGTT CTTTCTCTAG GTCTCAATGG TGTGTGTATA   1980
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GCGGGGTCTG ATACGGGTGG TTTTGAGCCC GCACGTACTG AGATTGGGGA GGAGAAATAT   2040
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AACCACTACG TCAAGAAGGA CAGGAAATGG TTCCAGGAAC CATACGCGTA CCCCAAGCAT   2160
CTTGAAACCC ATCCAGAGCT CGCAGATCAA GCATGGCTTT ACAAATCTGT TCTAGAAATT   2220
TGCAGATACT GGGTAGAGCT AAGATATTCC CTCATCCAGC TCCTTTACGA CTGCATGTTC   2280
CAAAACGTGG TCGATGGTAT GCCACTTGCC AGATCTATGC TCTTGACCGA TACTGAGGAT   2340
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ATCCTTGTAG CACCCATCCT CCACAGCCGT AACGAGGTTC CGGGAGAGAA CAGAGATGTC   2460
TATCTCCCTC TATTCCACAC CTGGTACCCC TCAAACTTGA GACCGTGGGA CGATCAGGGA   2520
GTCGCTTTAG GGAATCCTGT CGAAGGTGGC AGCGTTATCA ACTACACTGC CAGGATTGTT   2580
GCCCCAGAGG ATTATAATCT CTTCCACAAC GTGGTGCCGG TCTACATCAG AGAGGGTGCC   2640
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TTCAATATCT ACCCTGGAAA GGACAAGGAG TATGTGACGT ACCTTGATGA TGGTGTTAGC   2760
CGCGATAGTG CACCAGATGA CCTCCCGCAG TACCGCGAGG CCTATGAGCA AGCGAAGGTC   2820
GAAGGCAAAG ACGTCCAGAA GCAACTTGCG GTCATTCAAG GGAATAAGAC TAATGACTTC   2880
TCCGCCTCCG GGATTGATAA GGAGGCAAAG GGTTATCACC GCAAAGTTTC TATCAAACAG   2940
GAGTCAAAAG ACAAGACCCG TACTGTCACC ATTGAGCCAA AACACAACGG ATACGACCCC   3000
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AGCATCGTCG ATGTGAGCCA GGCGACCGTG AACATCGAGG GCGGGGTGGA ATGCGAAATT   3120
TTCAAGAACA CCGGCTTGCA TACGGTTGTA GTCAACGTGA AAGAGGTGAT CGGTACCACA   3180
AAGTCCGTCA AGATCACTTG CACTACCGCT TAG                                3213
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SEQ. ID. NO. 11

SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 3279 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

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                10         20         30         40         50         60
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   1 ATGTTTCCTA CCCTGACCTT CATAGCGCCC AGCGCGCTGG CCGCCAGCAC CTTTGTGGGC
  61 GCGGATATCC GATCGGGCAT TCGCATTCAA TCCGCTCTTC CGGCCGTGCG CAACGCTGTG
 121 CGCAGGAGCA AACATTACAA TGTATCCATG ACCGCATTGT CTGACAAGCA AACCGCTATC
 181 AGTATTGGCC CTGACAATCC GGACGGTATC AACTACCAAA ACTACGATTA CATCCCTGTA
 241 GCGGGCTTTA CGCCCCTCTC CAACACCAAC TGGTATGCTG CCGGCTCTTC CACTCCGGGC
 301 GGCATCACCG ACTGGACCGC TACCATGAAT GTCAAATTCG ACCGCATTGA CAATCCGTCG
 361 TACTCCAATA ACCATCCTGT TCAGATTCAG GTCACGTCGT ACAACAACAA CAGCTTCAGG
 421 ATTCGCTTCA ACCCTGATGG CCCCATTCGT GACGTCTCTC GAGGACCTAT CCTGAAACAG
 481 CAACTCACTT GGATTCGAAA CCAGGAGCTG GCGCAGGGAT GTAATCCGAA CATGAGCTTC
 541 TCTCCTGAAG GTTTTTTGTC TTTTGAAACC AAAGACCTAA ACGTTATAAT CTACGGCAAC
 601 TGCAAGATGA GAGTCACGAA GAAGGATGGC TACCTCGTCA TGGAGAATGA CGAGTGCAAC
 661 TCGCAATCAG ATGGCAATAA GTGTAGAGGA TTGATGTACG TTGACCGGCT ATACGGTAAT
 721 GCTATTGCTT CCGTACAAAC GAATTTTCAC AAAGACACTT CTCGGAACGA GAAATTCTAT
 781 GGTGCAGGTG AAGTCAACTG TCGCTATGAG GAGCAGGGTA AGGCGCCGAC TTATGTTCTA
 841 GAACGCTCTG GACTCGCCAT GACCAATTAC AATTACGACA ACTTGAACTA CAACCAACCA
 901 GACGTCGTTC CTCCAGGTTA TCCCGACCAT CCCAACTACT ACATTCCAAT GTACTACGCA
 961 GCACCGTGGT TGGTCGTTCA GGGATGCGCG GGGACATCGA AGCAATACTC GTACGGTTGG
1021 TTTATGGACA ATGTCTCTCA GTCGTACATG AACACTGGAG ATACGGCGTG GAACTGCGGA
1081 CAGGAAAACC TGGCATACAT GGGCGCGCAA TACGGGCCAT TTGATCAGCA CTTTGTGTAT
1141 GGTGATGGAG ATGGCCTTGA AGATGTCGTC AAAGCGTTCT CCTTTCTTCA AGGAAAGGAG
1201 TTCGAAGACA AAAAACTCAA CAAGCGTTCT GTAATGCCTC CGAAGTACGT GTTTGGTTTC
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1321 AACATCTCAG TGCAAGAGAT TGTGGAGGGT TACCAGGATA ACGACTACCC CTTTGAAGGG
1381 CTCGCGGTAG ATGTTGATAT GCAAGATGAT CTGCGAGTGT TTACTACCAA ACCAGAATAT
1441 TGGTCGGCAA ACATGGTAGG CGAAGGCGGT GATCCTAATA ACAGATCAGT CTTTGAATGG
1501 GCACATGACA GGGGCCTTGT CTGTCAGACG AACGTAACTT GCTTCTTGAG GAACGATAAC
1561 AGTGGGAAAC CATACGAAGT GAATCAGACA TTGAGGGAGA AACAGTTGTA TACGAAGAAT
1621 GATTCCTTGA ACAACACCGA TTTTGGAACT ACCTCGGATG GGCCTGGCGA TGCGTACATT
1681 GGACATTTGG ACTATGGTGG TGGAGTGGAG TGTGATGCAA TCTTCCCAGA CTGGGGTCGA
1741 CCAGACGTGG CTCAATGGTG GGGAGAAAAC TACAAGAAGC TGTTCAGCAT TGGTCTCGAT
1801 TTCGTGTGGC AGGATATGAC GGTACCTGCG ATGATGCCGC ACCGACTCGG TGATGCTGTC
1861 AACAAAAATT CCGGTAGTTC GGCGCCGGGC TGGCCGAATG AGAACGATCC ATCCAACGGA
1921 CGATACAACT GGAAATCTTA TCATCCGCAA GTGCTCGTGA CCGACATGCG CTATGGTGCA
1981 GAGTATGGAA GGGAACCGAT GGTGTCTCAA CGCAACATTC ACGCCTACAC TCTTTGTGAA
2041 TCTACCAGAC GGGAGGGAAT TGTGGGAAAC GCAGACAGTT TGACCAAGTT CCGCCGCAGT
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2101 TACATCATCA GTCGAGGAGG TTACATCGGT AACCAGCATT TCGGAGGGAT GTGGGTTGGG
2161 GACAACAGTG CCACAGAATC CTACCTCCAA ATGATGTTGG CGAACATTAT CAACATGAAT
2221 ATGTCGTGCC TCCCGCTAGT TGGCTCTGAT ATTGGCGGGT TCACCCAGTA CAATGATGCG
2281 GGCGACCCAA CCCCCGAGGA TTTGATGGTA AGATTCGTGC AGGCTGGCTG TCTGCTACCG
2341 TGGTTCAGAA ACCACTATGA CAGGTGGATT GAGTCCAAGA AGCACGGGAA GAAATACCAG
2401 GAGTTATACA TGTACCCGGG GCAAAAGGAT ACGTTGAAGA AGTTCGTTGA ATTCCGCTAC
2461 CGCTGGCAGG AGGTTTTGTA CACAGCCATG TACCAAAATG CTACCACTGG AGAGCCGATC
2521 ATCAAGGCGG CGCCCATGTA CAACAACGAC GTCAACGTGT ATAAATCGCA GAATGATCAT
2581 TTCCTTCTCG GTGGACATGA CGGCTATCGT ATTCTCTGCG CACCTGTTGT GCGCGAAAAT
2641 GCGACAAGTC GCGAAGTGTA CCTGCCTGTG TATAGCAAGT GGTTCAAATT CGGACCGGAC
2701 TTTGACACTA AGCCCTTGGA AAATGAGATT CAAGGAGGTC AGACGCTTTA TAATTACGCT
2761 GCACCGCTGA ACGATTCGCC GATATTTGTG AGGGAAGGGA CTATTCTTCC GACACGGTAC
2821 ACGCTGGACG GTGTGAACAA ATCTATCAAC ACGTACACAG ACAATGATCC GCTTGTATTT
2881 GAGCTGTTCC CTCTCGAAAA CAACCAGGCG CATGGCTTGT TCTATCATGA TGATGGCGGT
2941 GTCACCACCA ACGCTGAAGA CTTTGGCAAG TATTCTGTGA TCAGTGTGAA GGCCGCGCAG
3001 GAAGGTTCTC AAATGAGTGT CAAGTTTGAC AATGAAGTTT ATGAACACCA ATGGGGAGCA
3061 TCGTTCTATG TTCGTGTTCG TAATATGGGT GCTCCGTCTA ACATCAACGT ATCTTCTCAG
3121 ATTGGTCAAC AGGACATGCA ACAGAGCTCC GTGAGTTCCA GGGCGCAAAT GTTCACTAGT
3181 GCTAACGATG GCGAGTACTG GGTTGACCAG AGCACGAACT CGTTGTGGCT CAAGTTGCCT
3241 GGTGCAGTTA TCCAAGACGC TGCGATCACT GTTCGTTGA

SEQ. ID. NO. 12

SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 1712 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

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61 CATGATTCAG ATCCTGACTA CTATATTCCG ATGTACTTTG CGGCACCATG GGTGATCGCA
121 CATGGATATC GTGGCACCAG CGACCAGTAC TCTTATGGAT GGTTTTTGGA CAATGTATCC
181 CAGTCCTACA CAAACACTGG CGATGATGCA TGGGCTGGTC AGAAGGATTT GGCGTACATG
241 GGGGCACAAT GTGGGCCTTT CGATCAACAT TTTGTGTATG AGGCTGGAGA TGGACTTGAA
301 GACGTTGTGA CCGCATTCTC TTATTTGCAA GGCAAGGAAT ATGAGAACCA GGGACTGAAT
361 ATACGTTCTG CAATGCCTCC GAAGTACGTT TTCGGATTTT TCCAAGGCGT ATTCGGAGCC
421 ACATCGCTGC TAAGGGACAA CTTACCTGCC GGCGAGAACA ACGTCTCTTT GGAAGAAATT
481 GTTGAAGGAT ATCAAAATCA GAACGTGCCA TTTGAAGGTC TTGCTGTGGA TGTTGATATG
541 CAAGATGACT TGAGAGTGTT CACTACGAGA CCAGCGTTTT GGACGGCAAA CAAGGTGGGG
601 GAAGGCGGTG ATCCAAACAA CAAGTCAGTG TTTGAGTGGG CACATGACAG GGGCCTTGTC
661 TGCCAGACGA ATGTAACTTG CTTCTTGAAG AACGAGAAAA ATCCTTACGA AGTGAATCAG
721 TCATTGAGGG AGAAGCAGTT GTATACGAAG AGTGATTCCT TGGACAACAT TGATTTTGGA
781 ACTACTCCAG ATGGGCCTAG CGATGCGTAC ATTGGACACT TAGACTACGG TGGTGGTGTG
841 GAGTGTGATG CACTATTCCC AGACTGGGGT CGACCAGACG TGGCTCAATG GTGGGGCGAT
901 AACTACAAGA AACTATTCAG CATTGGTCTC GATTTCGTCT GGCAAGATAT GACGGTACCT
961 GCGATGATGC CGCACCGACT CGGTGACCCT GTCGGCACAA ATTCCGGTGA GACGGCGCCG
1021 GGCTGGCCGA ATGATAAGGA TCCATCCAAC GGACGATACA ATTGGAAGTC TTACCATCCG
1081 CAAGTGCTCG TGACTGACAT GAGGTATGAC GATTACGGAA GAGATCCCAT TGTTACGCAA
1141 CGCAATCTCC ATGCCTACAC TCTTTGTGAG TCTACTAGGA GGGAAGGCAT TGTTGGAAAC
1201 GCAGATAGTC TGACGAAGTT CCGCCGCAGC TATATTATCA GTCGTGGAGG CTACATCGGT
1261 AATCAGCACT TTGGTGGGAT GTGGGTAGGA GACAACTCTT CTACGGAAGA CTACCTCGCA
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1381 ATTGGAGGTT TCACGGAGCA TGACAAGAGA AACCCTTGCA CACCGGACTT GATGATGAGA
1441 TTTGTGCAGG CTGGATGCTT GCTACCGTGG TTCAGGAACC ACTACGATAG GTGGATCGAG
1501 AGCAAGAAAC ACGGAAAGAA CTACCAAGAG TTGTACATGT ACCGCGACCA CTTGGACGCC
1561 TTGAGAAGTT TTGTGGAACT CCGCTATCGC TGGCAGGAAG TGTTATACAC AGCCATGTAT
1621 CAGAATGCTT TGAACGGGAA GCCGATCATC AAAACGGTCT CCATGTACAA CAACGATATG
1681 AACGTCAAAG ATGCTCAGAA TGACCACTTC CT

CLAIMS

1. A process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques.

2. A process according to claim 1, wherein the glucan comprises $\alpha$-1,4 links.

3. A process according to claim 1 or claim 2 wherein the glucan is starch.

4. A process according to any one of the preceding claims wherein the glucan is a substrate for a recombinant enzyme such that contact of the glucan with the recombinant enzyme yields the anti-oxidant.

5. A process according to any one of claims 1 to 4, wherein the enzyme is a glucan lyase.

6. A process according to claim 5, wherein the enzyme is an $\alpha$-1,4-glucan lyase.

7. A process according to claim 6, wherein the enzyme comprises any one of the sequences shown as SEQ ID Nos 1-6, or a variant, homologue or fragment thereof.

8. A process according to claim 7, wherein the enzyme is any one of the sequences shown as SEQ ID Nos 1-6.

9. A process according to any one of claims 5 to 8, wherein the enzyme is encoded by a nucleotide sequence comprising any one of the sequences shown as SEQ ID Nos 7-12, or a variant, homologue or fragment thereof.

10. A process according to claim 9, wherein the enzyme is encoded by a nucleotide sequence having any one of the sequences shown as SEQ ID Nos 7-12.

11. A process according to any one of the preceding claims, wherein the anti-oxidant is anhydrofructose.

12. A process according to claim 11, wherein the anti-oxidant is 1,5-D-anhydrofructose.

13. A process according to any one of the preceding claims, wherein the medium is, or is used in the preparation of, a foodstuff.

14. A process according to claim 13, wherein the foodstuff is a beverage.

15. A process according to claim 14, wherein the beverage is an alcoholic beverage.

16. A process according to claim 14, wherein the beverage is a wine.

17. A process according to any one of the preceding claims, wherein the anti-oxidant is prepared *in situ* in the component and is then released into the medium.

18. A process according to any one of the preceding claims, wherein the component is a plant or a part thereof.

19. A process according to claim 18, wherein the component is all or part of a cereal or a fruit.

20. A process according to claim 20, wherein the component is all or part of a grape.

21. A process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared by use of a recombinant glucan lyase.

22. A process according to claim 21 wherein the glucan lyase is that as defined in any one of claims 6 to 10.

23. A medium prepared by the process according to any one of the preceding claims.

24. Use of anhydrofructose as an anti-oxidant for a medium comprising at least one other component, wherein the anhydrofructose is prepared *in situ* in the medium.

25. Use of anhydrofructose as a means for imparting or improving stress tolerance in a plant, wherein the anhydrofructose is prepared *in situ* in the plant.

26. Use of anhydrofructose as a means for imparting or improving the transformation of a grape, wherein the anhydrofructose is prepared *in situ* in the grape.

27. Use of glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the glucan lyase is prepared *in situ* in the plant.

28. Use of glucan lyase as a means for imparting or improving the transformation of a grape, wherein the glucan lyase is prepared *in situ* in the grape.

29. Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the nucleotide sequence is expressed *in situ* in the plant.

30. Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving the transformation of a grape, wherein the nucleotide sequence is expressed *in situ* in the grape.

31. A process or medium substantially as described herein.

FLH Docket No.
674509-2020

# DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

(Includes reference to PCT International Applications)

FROMMER LAWRENCE & HAUG, LLP
File No.: 674509-2020

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural, names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention ENTITLED:

## A PROCESS FOR PREPARING AN ANTI-OXIDANT

the specification of which:

X is attached hereto
was filed on ________as:
United States Application Serial No.____________
X is National Phase of PCT Application No. PCT/IB98/00708, filed May 6, 1998 and designating the U.S., published as WO/98/50532.
X with amendments through DATE EVEN HEREWITH (if applicable, give details).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 (a) - (d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application(s) designating at least one country other than the United State of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT International applications designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign/PCT Application(s) [list additional applications on separate page]:

| Country (or PCT) | Application Number: | Filed (Day/Month/Year) | Priority Claimed: Yes | Priority Claimed: No |
|---|---|---|---|---|
| GREAT BRITAIN | 9709161.5 | 06/MAY/1997 | X | |
| INTERNATIONAL BUREAU PCT | PCT/IB98/00708 | 06/05/1998 | X | |



SJL1900

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

__________ ______
(Application Number) (Filing Date)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or § 365 (c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Prior U.S. (or U.S.-designating PCT) Application(s) [list additional applications on separate page]:

| U.S. Serial No.: | Filed (Day/Month/Year) | PCT Application No. | Status (patented, pending, abandoned) |
|---|---|---|---|
| | 06/05/98 | PCT/IB98/00708 | PENDING (this is the National Phase) |

I hereby appoint Thomas J. Kowalski, Registration No. 32,147, and FROMMER LAWRENCE & HAUG, LLP or their duly appointed associates, my attorneys or agents, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therewith, and to insert the Serial Number of the application in the space provided above, and specify that all communications about the application are to be directed to the following correspondence address:

THOMAS J. KOWALSKI, Esq.
c/o FROMMER LAWRENCE &
HAUG LLP
745 Fifth Avenue
New York, NY 10151
FAX (212) 588-0500

Direct all telephone calls to: (212) 588-0800
to the attention of:
Thomas J. Kowalski, Esq.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FLH Docket No.
674509-2020

INVENTOR(S):

Signature: ______________________________ Date: ______________

Full name of sole or first inventor: BUCHTER-LARSEN, Aksel
Residence: Amager Felledves 27, DK-2300, Copenhagen, DENMARK
Citizenship: Danish

Signature: ______________________________ Date: ______________

Full name of 2nd joint inventor (if any): MARCUSSEN, Ian
Residence: Knabrostrede 25, DK-1210, Copenhagen, DENMARK
Citizenship: Danish

Post Office Address(es) of inventors [if different from residence]:

NOTE: In order to qualify for reduced fees available to Small Entities, each inventor and any other individual or entity having rights to the invention must also sign an appropriate separate "Verified Statement (Declaration) Claiming [or Supporting a Claim by Another for] Small Entity Status" form [e.g. for Independent Inventor, Small Business Concern, Nonprofit Organization, Individual Non-Inventor].



SJL1900